Variation in Joint Fluid Composition and Its Effect on the Tribology of Replacement Joint Articulation

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SUBMITTED TO THE HARVARD-MIT DIVISION OF HEALTH SCIENCES AND TECHNOLOGY
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY IN MEDICAL ENGINEERING
AT THE
MASSACHUSETTS INSTITUTE OF TECHNOLOGY

SEPTEMBER 2003

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ABSTRACT

Polyethylene wear is a significant clinical problem limiting the long-term survival of joint replacement prostheses, particularly in total hip arthroplasty (THA) and total knee arthroplasty (TKA). Although the tribology of joint replacement has consequently become an area of significant research, the effect of joint fluid on lubrication in the replaced joint has been largely overlooked. Several factors that affect the tribology of metal on polyethylene articulation in joint prostheses stem from the fluid lubricating the joint. In particular, the properties and composition of joint fluid likely contribute to fluid film lubrication and boundary lubrication in joint replacements, as they do in natural joints. The primary objective of this thesis is to examine the effect of natural variation in joint fluid composition and properties on friction, lubrication, and wear in joint arthroplasty.

To achieve this goal, several parameters relating to the composition and mechanical properties of joint fluid are determined. Steady shear viscosity and linear viscoelastic properties of joint fluid are evaluated as indicators of its mechanical properties. Furthermore, concentrations of the hyaluronic acid, protein, and phospholipid in joint fluid are measured using standard biochemical techniques. The molecular weight of hyaluronic acid is also determined using size exclusion chromatography. These properties and components are evaluated in joint fluid from patients undergoing TKA and from patients undergoing surgical revision of an existing TKA (as well as from other patient groups). Results are considered in the context of previous studies of healthy and diseased synovial fluid. Correlations between and among components and flow properties are determined.

Friction tests are performed on articulations between ultra-high molecular weight polyethylene (PE) and cobalt-chromium-molybdenum alloy (Co-Cr), materials commonly used in total joint replacement prostheses. These tests evaluate joint fluid samples as well as synthetic joint lubricants that are composed based on the range of compositions and properties determined. Certain components are found to increase friction in this articulation relative to water lubrication, but some joint fluid samples performed as well as bovine serum. Significant differences in tribology demonstrated by these experiments indicate that the composition of joint fluid affects the tribology of Co-Cr on PE joint prostheses, though the variability in friction could not be explained by physiological variation in the components examined. In related work, the relative importance of contact area and normal load is evaluated in the wear of a Co-Cr on PE articulation. Within a relevant range of contact stress, volumetric wear rate increased with increasing contact area, and was independent of normal load. The results of these tribological investigations are brought together in a conceptual framework under which to consider the wear of PE in TJA.

ACKNOWLEDGEMENTS

I would like to begin by acknowledging God, who makes all things possible, and thanking Him for the opportunity to pursue and complete graduate study. Second, I thank my wife, Betsy, for her unwavering love and support, and for editing the final draft. Third, I thank my advisor, Professor Spector, for direction, support, and flexibility. I thank the administrators and faculty of the Division of Health Sciences and Technology, and the faculty of Harvard Medical School, for their commitment to teaching excellence and for the great care they have for their students. I thank Professors Ortiz, Cohen and McKinley for serving on my thesis committee.

In addition to these key figures, a number of people directly contributed to particular aspects of the research in this thesis. With regard to the rheological work, I would like to express my gratitude to the members of the former Non-Newtonian Fluids Lab who have helped my research: Shelley Anna, Jonathan Rothstein, Daryl Overby, and Pirouz Kavehpour. With regard to the composition work, I am thankful to Michael Wilson at EIC Labs for his willingness to lend equipment, as well as to Shawn Kinney and Andrea Wagner at Hyaluron, Inc., for their expertise. With regard to the friction experiments, I appreciate Professor Saka's lively commentaries on life and tribology. I also thank Yoon Noh for helping me set up the experimental apparatus. With regard to the wear experiments, both Anuj Bellare and Marybeth Turrell were kind to permit my use of the Orthopaedic Research Laboratory wear tester. I acknowledge valuable discussions relating to the wear findings with Victoria Good and Will Sauer. Additionally, I thank Tim McClure of the Center for Materials Science and Engineering and Yin-Lin Xie of the Department of Materials Science and Engineering for their help with profilometry and polishing, respectively.

To conclude my professional acknowledgements, I would like to acknowledge my sources of funding and materials. Funding was provided by the Brigham Orthopaedic Foundation, the Biokinetix Foundation, the VA Boston Healthcare System, and the Harvard-MIT Division of Health Sciences and Technology. HA supplements were provided by Anika Therapeutics and Smith & Nephew Orthopaedics. Most joint fluid samples were obtained by Richard Scott of New England Baptist Hospital and Robert Schaefer of Baw Beese Sports Medicine & Joint Care.

Additionally, I would like to acknowledge a few people who have blessed my graduate school experience even though they did not contribute directly to the work of the thesis. I thank my predecessor as senior student in the Orthopaedic Research Laboratory, Cyndi Lee, who exemplified efficiency in her work. I specially thank Marybeth Turrell for her friendship during her time here and for keeping me sane. I am grateful for the qualifying examination coaching offered by Eric Osborn and Jonathan Thierman. Finally, I would like to thank those who motivated me to enter graduate study and encouraged me to press on with all speed. Thank you, Professor Lee Lynd, for encouraging me to find the best path for me. Thank you, Alex and Chris Hartemink, for introducing me to HST. Thank you, Shannon Walsh, for beating me in the race for a doctorate, but only by one year.

This thesis is dedicated to my grandfather, Francis Angelo Mazzucco, who was always my biggest fan.

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ABBREVIATIONS

Certain abbreviations used only in the appendices are excluded.

Abbreviation	Term	First Reference
ANOVA	analysis of variance	3.5.2
ASTM	American Society for the Testing of Materials	2.4.3
Co-Cr	cobalt-chromium-molybdenum alloy	1.2.3
DPPC	L-α-dipalmatoyl phosphatidylcholine	2.2.4
EDTA	ethylene-diaminetetraacetic acid	2.4.3
EHD	elastohydrodynamic	2.1.1
HA	hyaluronic acid	2.1.1
OA	osteoarthritis	1.2.1
Ox-Zr	oxidized zirconium	5.0
PE	ultrahigh molecular weight polyethylene	1.1
POF	pin-on-flat	2.4.4
PBS	phosphate buffered saline	5.6.1
PTFE	poly tetrafluoroethylene	2.4.2
RA	rheumatoid arthritis	1.2.2
SAPL	surface-active phospholipids	2.2.4
SEC	size exclusion chromatography	2.2.3
SEM	scanning electron microscope *or* microscopy	2.4.3
THA	total hip arthroplasty	1.1
TJA	total joint arthroplasty	1.1
TKA	total knee arthroplasty	1.1

FIGURES

FIGURES (CONTINUED)

SELECTED EQUATIONS

SYMBOLS

Certain symbols used within a single discussion or only as arbitrary constants are excluded.

Symbol	Description	Units
a	Hertzian contact radius	m
a_C	Concentration shift factor	dimensionless
a_I	Ionic strength shift factor	dimensionless
A	Area	m^2
A_a	Apparent area of contact	m^2
A_l	Lubricated area of contact	m^2
A_r	Real contact area	m^2
c	Consistency (in the Cross model)	s
C	Wear efficiency	dimensionless
C	Calibration constant	dimensionless
d	Rate index (in the Cross model)	dimensionless
d	Sliding distance	m
f_c	Crossover frequency	Hz
E_{PE}	Young's modulus of polyethylene	Pa
E'	Reduced modulus	Pa
F	Force	N
F_0	Force amplitude	N
F_{spring}	Force borne by the elastic nature of a viscoelastic fluid	N
F_{damper}	Force borne by the viscous nature of a viscoelastic fluid	N
G_c	Modulus at crossover frequency	Pa
G'	Storage modulus	Pa
G'_f	Storage modulus at frequency f	Pa
G''	Loss modulus	Pa
G''_f	Loss modulus at frequency f	Pa
h	Gap between plates in Couette flow	m
H	Hardness	Pa
k	Clinical wear factor	mm^3/Nm
k'	Revised clinical wear factor	mm^3/Mcycle, or dimensionless
K	Power law coefficient	Pa s^n
l	Sliding distance	m
M_n	Molecular weight, number average	Da
M_p	Molecular weight, peak average	Da
M_v	Molecular weight, viscosity average	Da
M_w	Molecular weight, mass average	Da
M_z	Molecular weight, z-average	Da
n	Power law index	dimensionless
N_1	First normal stress difference	Pa

SYMBOLS (CONTINUED)

Symbol	Description	Units
R	Radius	m
R'	Reduced radius	m
R^2	Correlation coefficient	dimensionless
R_a	Average roughness	m
V	Volume	m^3
V_0	Steady velocity of top plate in Couette flow	m/s
W	Normal Load	N
α	Pressure-viscosity coefficient	Pa^{-1}
α	Chance of false positive in evaluating null hypothesis	dimensionless
β	Chance of false negative in evaluating null hypothesis	dimensionless
γ	Shear strain	dimensionless
γ_0	Shear strain amplitude	dimensionless
$\dot{\gamma}$	Shear rate	s^{-1}
η	Steady shear viscosity	Pa s
η'	Dynamic viscosity	Pa s
η_0	Low shear rate viscosity	Pa s
η_0	In piezoviscosity, the viscosity at atmospheric pressure	Pa s
η_{1Pa}	Viscosity at 1 Pascal shear stress	Pa s
η_∞	High shear rate viscosity	Pa s
η_{Max}	Maximum measured viscosity (typically at minimum shear stress)	Pa s
η_p	Viscosity at elevated pressure p	Pa s
$[\eta]$	Intrinsic viscosity	ml/g
θ	Angle of asperity	dimensionless
μ	Coefficient of friction	dimensionless
μ_d	Mean coefficient of dynamic friction	dimensionless
μ_s	Coefficient of maximum static friction	dimensionless
υ_{PE}	Poission's ratio of PE	dimensionless
σ	Shear stress	Pa
τ_l	Stress required to shear a boundary lubricant	Pa
τ_w	Shear stress associated with plowing or adhesive wear	Pa
ϕ	Phase angle	dimensionless
ω	Angular velocity	rad/sec

TABLES

CHAPTER 1

INTRODUCTION

1.1 Significance of the Research

The research described in the succeeding chapters primarily analyzes joint fluid in the context of its effect on tribology in total joint arthroplasty (TJA). Particular emphasis is placed on the effect of joint fluid on total knee arthroplasty (TKA) and total hip arthroplasty (THA). This emphasis has been chosen because of the importance of wear-related issues in TKA and THA due to the high stresses in their components relative to other joint replacement articulations. In particular, the articulation of ultrahigh molecular weight polyethylene (PE) on metal, leading to the generation of PE wear particles, has become an issue of significant concern in orthopaedic research. Nonetheless, this research is also relevant for other combinations of materials for use in the hip and knee, as well as other implant articulations.

1.1.1 Rationale behind the Research

TJA has become an important surgical intervention in the last 40 years, but its success has been limited by tribological failure. PE wear, leading to aseptic (non-infectious) loosening, has become a prominent problem in TJA, placing unfortunate limits on this otherwise very successful surgical treatment. In particular, as the patient population in need of treatment for joint disease continues to grow younger and more active, the relatively higher demands on the prosthesis lead to increased risk of wear-related failure. Within the arena of replacement joint tribology, one question that has largely gone unanswered is what factors intrinsic to the patient, outside of behavioral ones, contribute to wear. The variability of wear rates encountered *in vivo* suggests the potential importance of intrinsic patient factors to the tribology of joint prostheses. Furthermore, the wide variation in quantity and flow properties of joint fluid found in preliminary studies has suggested that the joint lubricant is a factor worthy of examination in the tribology of TJA.

1.1.2 Benefits of the Research

Understanding the role of individual components in the lubrication of joint prostheses is the first step in improving the treatment of joint diseases. For example, it would be desirable to be able to assay a patient's synovial fluid before surgery to determine the quality of their joint fluid. Such an assay could help surgeons and patients decide whether TJA is the best option for the patient. Patients could expect better outcome prediction based upon analysis of their synovial fluid.

Second, knowledge of the components of joint fluid in TJA and their contribution to joint tribology would lead to better materials selection, and more pointed evaluation techniques for potential prosthetic materials. A joint simulator lubricant that truly represents the behavior of the joint fluid would make wear tests better at predicting the *in vivo* performance of the prosthesis.

Third, this research is a first step in building better therapies for osteoarthritis. Intra-articular injection to treat joint disease is not a new therapy. An understanding of the mechanisms by which it may protect the joint, however, would be a new development. Knowing what combination of joint fluid components best lubricates the replacement joint is a first step toward designing a pharmaceutical treatment to improve the natural lubrication of the replacement joint.

Finally, this research contributes toward an understanding of the steps leading to aseptic loosening and failure of TJA, particularly with respect to the effects of lubricant. This understanding will ultimately lead to better prosthesis design, hopefully eliminating generation of wear particles as a contributing factor in arthroplasty failure.

1.2 An Introduction to Synovial Joints and Arthroplasty

The human body prevents wear in synovial joints by a remarkable lubricating system. Articular cartilage and synovial fluid in the joint enable motion with a coefficient of friction less than 0.01.[1] These joints can function *in vivo* for 70 years with cartilage turnover outpacing wear. Consequently, the lubrication of synovial joints has been heavily investigated in the last fifty years; much of this study has focused on synovial fluid. Much more tribological research has been conducted regarding the synovial joint than the replacement joint, particularly with respect to the effect of lubricant. Therefore, the tribology of synovial joints is used as the basis on which to build an examination of the tribology of the replacement joint.

1.2.1 Osteoarthritis

Osteoarthritis (OA) describes a collection of non-inflammatory disorders affecting the synovial joints of the upper and lower extremities. OA occurs as the result of a long process of progressive articular cartilage degeneration in the joints, causing pain, stiffness, deformity, and instability. It can originate from joint trauma (also known as post-traumatic arthritis), bone misalignment, and/or prolonged misuse, and often takes decades to become symptomatic. Consequently, OA typically presents in older patients, and can be present in one or many joints.

The prevalence of symptomatic OA is estimated at about 10% among people 63 and older in the United States,[2] though some evidence of OA can be found in 80% to 90% of people over the age of 65.[3] These figures may vary worldwide based upon genetic or behavioral differences among cultures. Per 100,000 American adults, there are an estimated 200 cases of OA in the knee and 50 cases in the hip diagnosed each year.[2] Factors that put individuals at increased risk of OA include advanced age,[4] female gender,[4] obesity,[5] athletic injury,[2] and family history.

OA is a progressive disease. At first, pain is often relieved by non-steroidal anti-inflammatory drugs. Other therapies that seem to work in some cases, and which are touted to protect or regenerate cartilage, rather than just prevent pain, include glucocorticoids and viscosupplementation.[6] These treatments cannot ultimately stop the progression of OA, however, and many patients require TJA.

1.2.2 Incidence and Prevalence of Total Joint Arthroplasty

TJA is a surgical treatment for OA and other joint disorders affecting the knee and hip, such as post-traumatic arthritis and rheumatoid arthritis (RA). TJA is indicated in patients who have a particularly painful or dysfunctional knee joint with extensive loss of articular cartilage due to OA or other joint disease. As of 2001, about 267,000 TKAs and 275,000 THAs were performed in the U.S. annually;[7] this figure is on the rise. These surgeries are generally considered very successful, in that patients have a 90% chance of keeping the prosthesis for more than ten years.[8] Patients report better mobility, less joint

pain, and higher quality of life after arthroplasty. Consequently, an increasing number of younger patients choose knee or hip replacement every year.

1.2.3 Prosthetic Components

In knee replacement surgery, the surgeon removes a portion of the distal femur, typically replacing it with a metal component, often composed of a cobalt-chromium-molybdenum (Co-Cr) alloy. The surgeon also removes the proximal portion of the tibia, typically replacing it with a PE plateau, which fits into a metal casing mounted to the tibia. Finally, the patella is often shaved down, and a polyethylene button fixed onto it. In THA, a metal component typically replaces the femoral head and PE typically replaces the acetabular cup. Thus, in both cases, the convex surface is hard, and the concave surface soft. In addition to the most common Co-Cr on PE articulation, there are a number of different material combinations that have been or are in use worldwide in TKA. These include ceramic-on-ceramic, ceramic-on-polyethylene, and countless different variants of pretreated polyethylenes and metal alloys. Although materials selection is an area of substantial research in TJA, a complete treatment of materials choice lies outside the scope of this thesis. Furthermore, substantial variety in prosthesis geometry and mobility exists, but these, too, lie outside the scope of this thesis.

1.2.4 Response of the Synovial Membrane to Injury

During surgery, the menisci are sacrificed, along with the collateral ligaments and the anterior cruciate ligament, a portion of the synovial sac and fluid, and the articular cartilage. Depending on the implant and the condition of the patient, the posterior cruciate ligament may or may not be retained. Each component can be cemented in place, though often the metal components are press-fit without cement to encourage bonding to the remaining portions of the femur and tibia.[9]

Since joint replacement constitutes substantial trauma to the joint, previous work regarding the response of particular aspects of the joint to injury is particularly relevant here. In a classic paper from 1925, J. Albert Key studied the regeneration and repair of the synovial membrane in 24 rabbits after synovectomy.[10] He found that, after a period of clot formation and fibrous deposition, the synovial membrane appeared to recover fully in these animals within 60 days. Since then, a number of studies have explicitly or implicitly confirmed this finding regarding synovial membrane regeneration after synovectomy in humans. A recent reference to that effect is given by Ostergaard *et al*, who confirmed using magnetic resonance imaging that previously inflamed synovial membranes regenerated after removal; after 12 months, the regenerated membranes often showed signs of recurrent inflammation.[11] Whether this kind of regeneration occurs after TKA has not been demonstrated, to my knowledge. It is likely that some regeneration of the synovial membrane occurs after TKA, but the altered biomechanical environment of arthroplasty may affect the healing process.

1.2.5 Changes in Synovial Fluid after Joint Replacement

During total knee arthroplasty, the synovial membrane is damaged, and much of the synovial fluid lost. After surgery, a new joint capsule forms around the prosthetic joint, and new joint fluid lubricates the joint. Both boundary and fluid-film lubrication

may contribute to the tribology of the prosthetic joint as they do in the natural joint, though the relative contribution of each type of lubrication likely differs.

Several differences between the natural and replacement joint disfavor lubrication in the latter. First, fluid film lubrication in natural joints is supported by the properties of cartilage. The elasticity of cartilage contributes to elastohydrodynamic lubrication[12] and the porosity of cartilage may enable squeeze-film[13] and weeping[14] lubrication. Traditional surfaces used for joint replacement do not replicate these qualities, and these modes of lubrication cannot exist in current replacement joints. A few investigators have, with mixed results, pursued surfaces that better mimic the natural ones, but none have been successfully marketed.[15-17]

Second, synovial fluid is composed of plasma filtrate and products of Type B synoviocytes and superficial chondrocytes. When the synovial membrane is damaged during joint replacement, both filtration and molecular synthesis may be compromised. The repaired synovial membrane may not duplicate the original membrane, and may not contain the same quantity and morphology of synoviocytes (even if the gross appearance is restored). Furthermore, it is not clear what biomechanical feedback is lost in the synovium by replacing the articular cartilage with a prosthetic surface. Since this membrane is of primary importance in the production and filtration of joint fluid, the fluid lubricating the replacement joint may differ from that lubricating the natural joint. Inasmuch as the lubricating quality of synovial fluid depends on these elements, the lubrication of the replacement joint will suffer.

Finally, boundary lubrication involves interactions between surfaces and fluid components. The interaction between components of synovial fluid and cartilage (which, though not fully understood, enables excellent boundary lubrication) is unlikely to be matched by the interaction between the fluid present in the replacement joint capsule and the implant surfaces. Replacement joint materials currently marketed are not designed to interact with joint fluid, and any interaction with joint fluid, positive or negative, is incidental. Thus, for several reasons, lubrication in the replacement joint does not nearly match that found in the natural joint.

Prior to the present work, little has been published regarding joint fluid after joint replacement. Since one of the key hypotheses tested in this work is that joint fluid after arthroplasty differs from joint fluid before arthroplasty, a distinction is made between the two. The fluid surrounding the joint prior to arthroplasty is called "synovial fluid," keeping with tradition, whereas fluid in the joint after arthroplasty is termed "joint fluid." This distinction is carried through the text so as to avoid confusion.

1.3 Wear in Total Joint Arthroplasty

One of the limiting factors in joint replacement durability is wear. When metal rubs against PE, particles of PE are removed from the surface as wear debris. Synovial macrophages (Type A synoviocytes) respond to these foreign bodies in an effort to digest and remove them. Synovial macrophages release a variety of regulators, including interleukin-1β, which is linked both to inflammation and to bone resorption by osteoclasts.[18] Bone resorption leads to prosthesis loosening,[19] instability, and pain. Consequently, wear particle generation can lead to prosthesis failure even if the worn surface continues to bear the applied loads and provide normal joint mobility. For a

review of the problems associated with the biological response to wear debris, see Willert *et al.*[20]

Analysis of retrieved prostheses suggests that wear in replacement knees is often caused by pitting or delamination processes, whereas in replacement hips, adhesive and abrasive processes dominate.[21] This difference may be related to differing stress patterns present in the two joints. Finite element modeling suggests maximum stresses in the replacement knee of 40 MPa, as opposed to 15 MPa in the hip,[22] where the yield strength of implant grade PE typically lies in the 20 MPa range. The results of clinical wear studies are discussed in greater detail in the next chapter.

1.3.1 Scope of the Problem in Total Knee Arthroplasty

An estimated 22,000 TKAs require revision surgery annually, and PE wear and implant loosening are the two primary reasons for revision.[23] Even these numbers do not fully capture the magnitude of the problem of wear in TKA, however. In fact, while some less successful designs have led to revision rates as high as 33%,[24] others have reported no failures due to wear or osteolysis in ten years.[25] A survey conducted in 1994 considering many clinical studies found a 3% failure rate in TKA within four years due to mechanical failure or aseptic loosening.[26] The same study found that only 4% of patients underwent revision within four years for any reason. Another study reported ten-year survivorship of 95% and fifteen-year survivorship of 90%.[27]

Part of the reason failure rates are so low in TKA is that the orthopaedic community has been slow to employ TKA except in elderly, inactive patients due to fear of wear-related failure.[28] Even now, because surgeons are aware of the limitations of the implants, patients are forced to pursue a less active lifestyle. The American Academy of Orthopedic Surgeons discourages knee replacement patients from contact sports, skiing, tennis, and "vigorous" walking because they can lead to excessive wear. Some clinicians still recommend high activity due to the numerous overall health benefits associated with an active lifestyle.[8] Nonetheless, patients undergoing TKA must curtail some of their more strenuous behaviors to avoid the threat of wear-related complications. The trend toward younger, more active patients only serves to heighten this problem. Currently, long life expectancy and high activity are relative contraindications for TKA.[27] Unfortunately, the younger patients who require TKA tend to be the same patients who want to engage in these activities – the same activities that first brought them to the orthopedic surgeon. Furthermore, revision, when it is necessary, is a complicated surgery with substantial risk to the patient and uncertain prognosis (see Gravallese *et al.*[29] for an example). To solve this conundrum, much research in the area of joint replacement has been devoted to reducing wear of the PE component.

1.3.2 Scope of the Problem in Total Hip Arthroplasty

Prosthesis wear is also a major problem in THA. Wear as a cause of failure is better documented in THA than in TKA. THA has a longer history and the first hard on soft hip replacements failed due to large-scale wear. These early hip failures may have contributed to the relatively conservative attitudes toward TKA implantation that still affect standard of care today. Similar concerns also limit the utility of THA for younger, more active patients.[30] Despite a conservative clinical approach, wear is still the primary cause of failure in THA for many designs.[31] Furthermore, revision rates are much higher

among patients under 55 years old, and revision rates have not decreased meaningfully since 1980.[32]

The wear mechanisms in THA differ from those in TKA because both contact stresses[22] and articulation patterns[33] differ in the two systems. In the hip, articulation follows a multidirectional pattern, where a point on the femoral head traces a rectangular shape on the acetabular component. It has been found that the generation of wear particles using stresses encountered in the replacement hip requires the use of multidirectional motion.[34,35] The replacement knee produces different particle morphology than the replacement hip[36] with a largely linear motion,[33] facilitated by the substantially higher stresses encountered in the knee. The higher stresses in TKA suggested to pioneers in the field that wear would be a more significant problem in the knee than the hip.

The particle morphology in the replacement hip suggests primarily abrasive wear, whereas the morphology of knee replacement particles (often large and flaky) suggests a fatigue or delamination process. The differences in particle morphology are important because both mobility[37] and biological activity[38] of wear particles vary based on particle size. This further underscores the importance of understanding the wear mechanisms involved.

Despite these differences, there is much to be learned about this problem in each joint by studying the other. For example, PE wear has been studied extensively in hip simulators because the articulation of the hip joint is well-defined. In the knee, it is less clear which motions are most relevant, since different activities of daily living may bring about widely variable rolling, sliding, and twisting motions to the joint. Consequently, standards of measure have not been as well established for knee simulation as for hip simulation.[39] Therefore, it may be of benefit to consider the more well-defined case in order to understand the more complex case. Furthermore, even if different wear mechanisms occur in the replacement knee and hip joints, the same mode of lubrication could dominate in both cases, and the same molecules may be relevant to such lubrication. Therefore, although the present research on occasion employs one joint or the other (*e.g.*, joint fluid from TKA or the use of THA standards), these studies are intended to apply to both TKA and THA.

1.3.3 Why Does the Lubricant Matter?

Clinical findings draw attention to the possibility that the lubricant is an important factor in the tribology of TJA. For example, clinical studies have found huge variability in the amount of wear from patient to patient. This has been found in TKA,[33] but was first seen upon examination of implanted hip prostheses. Over a quarter of early metal-on-PE implants underwent less than 0.5 mm of linear wear in a decade.[40] The fact that some joints experience negligible wear and some experience substantial wear leads to the question of what differs between patients. There are several possible explanations for this disparity: poor quality control in materials, variation in surgical technique, and different articulating environments are a few of them. The two former issues have been addressed through the years by surgeons and orthopedic manufacturers, but the latter has yet to be addressed. Primary variables in the articulating environment that have not yet been evaluated include quality and quantity of lubrication in the joint space.

1.4 Outline of the Thesis

This chapter has provided a brief introduction to the problem of wear in joint replacement for the scientific reader new to the topic. The second chapter provides extensive background on the topic, including a complete literature review of joint fluid in joint replacement. This background is organized by topic, starting with the mechanisms of fluid film lubrication and boundary lubrication, specifically as they relate to prosthetic articulations, continuing with the composition and properties of joint fluid, and concluding with the development of tribological studies related to TJA.

Successive chapters discuss in detail the work that I have done to examine aspects of the problem of lubrication of joint prostheses. Such work includes: flow properties of joint fluid in TKA, composition of joint fluid in TKA, friction of metal-on-PE articulations using physiological lubricants, and the effects of certain parameters on PE wear rate. Although each chapter is self-contained, an effort is made at the start and finish of each chapter to relate the findings to gaps in current knowledge, as discussed in Chapter 2. The final chapter summarizes the major findings of my research, discusses a model to explain the role of joint fluid and other important parameters in the tribology of metal-on-PE articulation, and suggests which future experiments would be of greatest value. The final chapter also makes note of the practical application of my research to improve patient care and implant research.

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CHAPTER 2

BACKGROUND

This thesis combines study in a number of fields, including rheology, tribology, biomaterials, and biochemistry. As such, there are number of separate lines of research that have been followed in the literature. This chapter summarizes previous work related to each of these lines of research, and is divided into four main sections. The first section provides an overview of the two main lubrication mechanisms relevant to TJA. This section is followed by a detailed discussion of the components of synovial fluid. Third, literature regarding the rheological properties of synovial fluid is presented, with some attention given to the consequence of these properties on lubrication. Finally, the application of engineering tribology to TJA is discussed, including tribological experiments on replacement joint articulations.

Little work has been conducted that relates specifically to lubrication of joint replacement by joint fluid; in each of the four sections, knowledge gained in work intended for related fields is applied to the present thesis. Therefore, each section begins with the current research on natural synovial joints, synovial fluid, non-medical articulations, or general tribology of TJA, as dictated by the bulk of related research. The sections eventually discuss what studies relate more directly to replacement joints, joint fluid, and the role of lubricant in the tribology of TJA. Each section concludes with the application of current knowledge to this thesis and a summary of the gaps in current knowledge that this thesis intends to fill.

2.1 Lubrication of Synovial and Replacement Joints

Two common engineering modes of lubrication are fluid film and boundary layer lubrication. Fluid film lubrication involves the formation of a fluid film between surfaces to bear a load. The action of moving the lubricant out of the way of the moving surface requires work, and the viscosity, or resistance to flow, of the lubricant provides the capacity to bear the load of the moving surface. The success of fluid film lubrication, then, depends on both the bulk properties (*e.g.*, viscosity) of the lubricant and velocity of motion. Other factors affecting fluid film lubrication include surface geometry, surface roughness, and the quantity of lubricant.

When fluid film lubrication cannot support the load of articulation (due to high loads or low relative motion), surface roughness exceeds the gap between the surfaces, and asperities on the surfaces make contact. In boundary lubrication, a component of the lubricant adheres to the articulating surfaces, forming a coating one or a few molecules thick. Without boundary lubrication, the surfaces make direct contact, possibly resulting in adhesion as well as abrasive plowing and delamination of the softer material. Repeated asperity contacts of this type eventually lead to wear. Unlike fluid film lubrication, boundary lubrication does not rely on motion for load support. Thus, when conditions do not permit fluid film lubrication, surfaces rely on boundary lubrication for protection. Boundary lubrication typically leads to higher friction and wear rates than fluid film lubrication does.

When boundary lubrication succeeds, however, surfaces suffer less damage because molecules adsorbed to the surfaces make contact instead of the asperities. Possible mechanisms of adhesive wear, abrasive wear, and subsurface damage are discussed below in section 2.4.1. For now, it is sufficient to consider that adhesion is reduced by boundary lubrication because the physical apposition required for bonding can be prevented. Abrasive wear may be reduced by boundary lubrication as well

because work is done shearing off the lubricating layer rather than plowing or deforming the softer surface. The boundary layer is replenished when the denuded surface is again exposed to lubricant.

For a given geometry of articulation, the lubricant, load, and relative velocity of the surfaces influence what type of lubrication takes place. First, boundary lubrication supports higher loads than fluid film lubrication supports. Friction is relatively independent of load and velocity in boundary lubrication. Since individual components rather than bulk properties enable boundary lubrication, friction is independent of fluid viscosity. On the other hand, in fluid film lubrication, friction increases with velocity, viscosity, and the reciprocal of load. (The relationship between the two terms is linear under certain geometries when a Newtonian fluid is employed.) Therefore, by varying load, velocity, and viscosity, it is possible to determine whether a set of conditions gives rise to boundary or fluid film lubrication.

A common method of evaluating the mode of lubrication in a given articulation uses a Stribeck curve. The Stribeck curve (Fig 2.1.1) plots the coefficient of friction, μ, versus a variety of parameters, usually including a viscous term, a velocity, and a force load. For many metal-on-metal journal bearings, such a comparison has given rise to a single curve for many combinations of velocity, viscosity, and load.[1] The flat portion of the curve close to the ordinate represents the boundary regime, and the increasing portion of the graph represents the fluid-film regime. The portion of the curve between these regions is called the mixed regime, in which both modes of lubrication occur. The Stribeck curve nicely shows all three of these regimes of lubrication, though such a useful curve can only be generated under certain geometries. Note in Figure 2.2.1 below that a relative minimum in the Stribeck curve indicates the transition between mixed and fluid film lubrication.

In order to reach full fluid film lubrication, the quantity of fluid must enable the fluid-film thickness to exceed the roughness of the articulating surfaces to prevent asperity contact. Experimental evidence suggests fluid film thickness must be at least three times average roughness of the surfaces (R_a) to ensure full fluid film lubrication in replacement joint articulations.[2] The load supported by the fluid and the thickness of the film depend on the bulk properties of the lubricant and the velocity of relative motion.

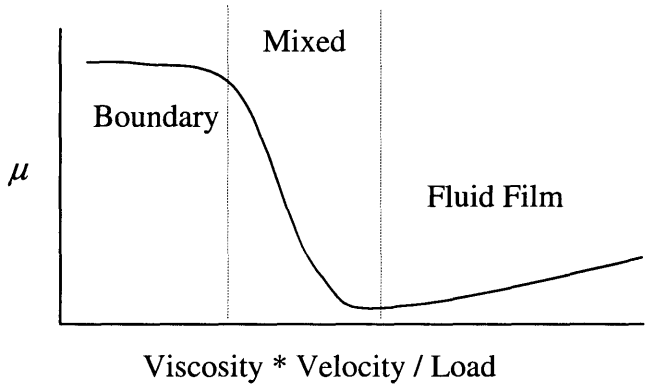

Fig. 2.1.1 Sample Stribeck curve This generic Stribeck curve shows three lubrication regimes. The relationship between μ and the given parameters show what type of lubrication is taking place.

The synovial joint has excellent tribological properties, including very low wear and a coefficient of dynamic friction of 0.01.[3] There has been some debate as to the relative importance of various lubrication mechanisms employed by the synovial joint. The very low dynamic coefficient of friction in synovial joints suggests fluid film lubrication, but certain aspects of the articulation suggest boundary lubrication. For example, fluid films require relative motion to provide support; during prolonged standing, fluid film lubrication is unlikely to occur in the load-bearing joints. Consequently, it is believed that both fluid film[4,5] and boundary[3] mechanisms contribute to the tribology of the synovial joint. The likelihood of multiple functioning modes of lubrication is also suggested by the fact that the same lubricating system appears to act in many joints under a variety of conditions: this despite the variety of different conditions prevailing in different joints during different activities. Further evidence for each mode of lubrication is discussed below.

Understanding the role of synovial fluid in synovial joint tribology is essential to understanding the role of joint fluid in replacement joint tribology. How these modes of lubrication occur in the replacement joint depends on the means by which they occur in the natural joint and on the differences between the natural environment and that created by joint replacement.

2.1.1 Fluid Film Lubrication

Prior to 1959, it was generally believed that synovial joints operated by fluid film lubrication, in part due to their exceedingly low coefficient of friction. John Charnley and other TJA pioneers questioned this assumption,[3] suggesting boundary lubrication in synovial joints. Soon, researchers began to critically examine lubrication in synovial joints, suggesting intermediate alternatives to the extremes of hydrodynamic and boundary lubrication. A review of this early work was given by Dintenfass.[6]

In 1976, the first modern study to suggest fluid film lubrication in synovial joints examined the lubrication of cartilage-on-glass using synovial fluid.[7] Fluid from patients with ligament and meniscal lesions lubricated better (*i.e.*, produced a lower coefficient of friction) than lower viscosity fluid from patients with degenerative joint diseases. The authors associated the increase in friction with a change from fluid film lubrication to mixed lubrication, as shown schematically above in the Stribeck curve. The conditions of the articulation favored boundary lubrication, however. Since fluid film lubrication was unlikely, some other factor besides viscosity may have caused the difference in friction between healthy and diseased joint fluid samples.

In 1978, O'Kelly *et al.* found that digesting synovial fluid with hyaluronidase (an enzyme that breaks the polymeric bonds in hyaluronic acid (HA) and thus reduces the viscosity of synovial fluid) reduced the lubricating ability of synovial fluid in a cadaveric hip.[5] Digestion by trypsin (a protease), on the other hand, did not affect friction. These tests were performed under dynamic loading conditions, and provided further evidence for a transition from fluid film lubrication to mixed lubrication in the synovial joint with a decrease in viscosity. These experiments were performed under dynamic loading; related work supported a mixed lubrication regime in static loading.

Shortly thereafter, Roberts *et al.* found evidence of viscosity-dependent lubrication in hip joints.[8] They repeated and confirmed the work of O'Kelly *et al.* over a

range of normal loads. Roberts *et al.* found that viscosity determined μ under low normal loads (100 to 300 N), but found that viscosity did not affect friction at high loads (1180 to 1470 N). Further, using synovial fluid and synthetic lubricants of viscosity less than 0.05 Pa s (which is below the lower limit found for steady shear viscosity at low shear rates in "normal" human synovial fluid), they found viscosity-independent lubrication, suggesting a mixed or boundary mechanism. Some synovial fluid samples from patients with diseased joints exhibit viscosity that would place lubrication in the boundary regime found by Roberts, suggesting that fluid film lubrication occurs in healthy joints (with more viscous synovial fluid) but not in diseased joints (with less viscous synovial fluid). Although their data could be fit to a Stribeck curve displaying a relative minimum μ, the variation in data was great. The authors did not report any statistical analysis, but examination of the data showed that the variation in the data prevented any substantial demonstration of a transition from mixed lubrication to fluid film lubrication.

The strongest evidence in favor of fluid film lubrication comes from histological analysis of loaded joints. In 1999, Clark and coworkers prepared histological samples of loaded rabbit knee joints by "plunge-freezing" them while under physiological loading conditions.[9] They found a uniform separation between cartilage layers of about 0.1 μm. This finding indicated that a fluid film separated the surfaces, but did not conclusively demonstrate the mechanism of fluid film lubrication. This study used rabbit knee joints, which bear lower loads than human knees and hips do. In 1980, Terayama *et al.* had conducted similar work on human hips and knees under load, and found gaps of 200 to 600 μm.[10] These gaps seem too large to be explained by fluid-films, however, and may have been an artifact of the preparation.

Other work in this field focused on what modes of fluid film lubrication are relevant in synovial joints. There are many variations of fluid film lubrication that reflect various levels of complexity that can occur in an articulation. As our understanding of the synovial joint has progressed, the complexity of fluid film models used to understand synovial joint lubrication has increased. Below are some of the many fluid film models that have been employed to understand lubrication of the synovial joint in the last half century. These are considered in the context of their likely relevance to TJA.

Hydrodynamic Lubrication

In this, the most basic kind of fluid film lubrication, a wedge of fluid forms such that surface movement squeezes fluid from the base of the wedge to its apex. Hydrodynamic lubrication was derived analytically, so the relationship between the coefficient of friction and given parameters is well-defined. Coefficient of friction increases with velocity and lubricant viscosity in hydrodynamic lubrication, whereas it decreases with normal load. Although μ increases with viscosity, μ is typically much lower than the 0.1 to 0.2 value found in boundary layer lubrication. Increasing the viscosity leads to a larger gap between the surfaces, ensuring minimal wear. It is typically estimated that fluid film thickness must be more than three times R_a to prevent any asperity contact.[1]

Hydrodynamic lubrication cannot explain the tribology of synovial joints. Under some conditions, loads up to five times body weight may be borne by the knee or hip joints. These loads are well above the limits of hydrodynamic lubrication. Hydrodynamic lubrication may occur in synovial joints bearing minimal loads, such as

interphalangeal joints, but does not occur under normal conditions in human hips and knees.

Hydrodynamic lubrication may be very important to the tribology of other soft tissue articulations in the joint, however. For example, the synovial membrane of the knee folds over itself when the joint bends. The folds rub against each other, and so generate friction. A number of authors have suggested that loads in this interaction are low enough to permit hydrodynamic lubrication.[11,12] Furthermore, these soft tissues are more highly innervated than articular cartilage, and could be a source of more joint pain than articular cartilage when not lubricated properly. Thus, hydrodynamic lubrication, while not likely the mode of lubrication of the load bearing joints, may be an important aspect of joint disease.

Elastohydrodynamic Lubrication

Elastohydrodynamic (EHD) lubrication is an extension of hydrodynamic lubrication such that the elasticity of the articulating surfaces and the piezoviscous nature of the lubricant contribute to the maintenance of a fluid film.[13] In 1972, Walker noted that the contact area between the femoral condyles and tibial plateau increased to 3.2 cm^2 within one second after loading. This contact area increased to 5.8 cm^2 within half an hour.[14] These findings indicate significant viscoelastic behavior in articular cartilage, and suggest EHD lubrication. The elastic nature of cartilage lends articulation in the synovial joint to this type of lubrication, though the nonlinear viscoelastic behavior of cartilage complicates quantitative analysis.

In the late 1970s, McCutchen argued that cartilage is too wavy for EHD lubrication.[15] In response, Dowson argued that EHD lubrication occurs on a smaller scale, which he called micro-elastohydrodynamic lubrication.[16] Some authors have argued that undulations in cartilage are squashed under load to create a fluid film,[17] or that the wavy character of articular cartilage is an artifact of histological preparation,[10] and micro-elastohydrodynamic lubrication need not be invoked to allow fluid film lubrication of the synovial joint.[18] The strongest evidence against McCutchen's argument (and thus in favor of EHD lubrication) was the Clark *et al.* work, since it showed a flat cartilage surface under load, indicating that undulations in articular cartilage *are* artifacts. This experiment offers strong evidence in favor of EHD lubrication in the natural rabbit knee, with cartilage smoothed under load.

Current joint replacement prosthesis design has not considered EHD lubrication, so the materials are much stiffer than the natural joint: the elastic modulus of PE is two orders of magnitude higher than that of articular cartilage (1 GPa[19] versus 10 MPa[20]), and metals and ceramics are much stiffer still (100 GPa or more). Thus, one would expect EHD lubrication to be greatly compromised in replacement joints.

Numerical analysis of EHD lubrication in THA by Mabuchi *et al.* predicted a minimum thickness of 2 μm, assuming a synovial fluid viscosity of 0.015 Pa s. When changing the viscosity to that of serum, the film thickness fell to 0.3 μm.[21] This finding suggests that EHD lubrication can occur in THA, since the roughness of implant materials is on the order of one-third of the gap thickness. This finding also confirms the importance of joint fluid properties to this mode of lubrication, since a less viscous lubricant did not maintain a sufficiently thick fluid film. Smaller gaps were found, on the

order of 100 nm, in metal-on-metal prostheses, suggesting that EHD lubrication would be more difficult to attain in these articulations.[22]

One would expect an even smaller gap in TKA than in THA, since the surfaces are less conforming. These nonconforming surfaces with current replacement joint materials could not maintain a fluid film with a gap less than 0.1 μm even if the metal component maintained its pre-implantation smoothness. If EHD lubrication takes place in TKA, it may be due to the piezoviscous nature of joint fluid. Interestingly, both groups performing EHD analysis of THA ignored piezoviscous changes to joint fluid. Work conducted examining this property in synovial fluid is discussed below in section 2.3.6.

Squeeze Films

As two surfaces approach each other in a viscous medium, they exert a force on one another. This force can prolong the separation between the surfaces. Reynolds first described and quantitatively analyzed this process in the 19th century.[13] In load-bearing joints, this effect may provide lubrication during the stance phase of gait.[21] We can quantitatively analyze this effect by approximating the human knee joint as two parallel plates separated by a gap. The Reynolds Equation for squeeze films[13] gives

$$\Delta t = \beta\left(\eta B^3 L/2W\right)\left(1/h_2^2 - 1/h_1^2\right), \qquad \textit{Equation 2.1.1}$$

where B and L are the width and length of each plate, W is the normal load, η is the lubricant viscosity, h_1 and h_2 are the separation of the surfaces at the start and finish of the calculation, β is a constant determined by B/L, and Δt is the time over which a gap is maintained.

Let us treat the femoral condyles and tibial plateau as 2 cm by 4 cm plates ($\beta = 0.633$) initially separated by 1 mm. If the synovial fluid had a viscosity of 1 Pa s, a 75 kg person standing on one leg would maintain a gap greater 0.1 μm for more than a week, by first approximation. Using this same equation, synovial fluid with a viscosity of 1 mPa s would maintain the gap for about ten minutes. More detailed theoretical analyses, such as that by Hlavacek,[23] also suggest that squeeze film lubrication can occur when the joint contains healthy synovial fluid, but can be compromised when synovial fluid is inviscid. There is also ample empirical support for squeeze film lubrication in an increase in friction with time in the articulation of cartilage-on-cartilage[24] and cartilage-on-glass.[25] This finding supports the idea that a squeeze film temporarily maintains a separation between the surfaces (and therefore keeps friction low).

There is still disagreement in the literature over the importance of this mode of lubrication.[18,26] The importance of squeeze films is likewise unknown in replacement joints, but probably does not figure prominently in present designs. Although the treatment above does not depend on the surfaces *per se*, the elasticity of cartilage allows two non-congruent surfaces to conform, increasing their contact area and reducing contact pressure, as in EHD lubrication. This effect is reduced significantly in the relatively stiff replacement joint designs currently employed.

Fluid Film Lubrication in TJA

Although many researchers have promoted the consideration of fluid film lubrication in prosthesis design,[27-30] no such design has been successfully marketed. The

relevance of fluid film lubrication in existing designs has been examined: some suggest that squeeze film or EHD lubrication is possible in THA when joint fluid is sufficiently viscous,[21] though the same lubrication in TKA is unlikely. Other studies suggest that load-bearing metal-on-PE prostheses operate in the mixed or boundary regime.[31-34] Some authors suggest that ceramic-on-ceramic[33,35] or metal-on-metal[36,37] hip prostheses can operate in the fluid film regime. In any case, fluid film lubrication is essential to consider in joint replacement because if future joint replacement designs can employ this mode of lubrication, wear can be largely prevented.

An important point to note is that all forms of fluid film lubrication relevant to joint replacement depend on the flow properties of the lubricant. As is discussed below in section 2.3, these properties are degenerate in many disease states. This degeneration may affect the function of lubrication prior to arthroplasty and well as after arthroplasty.

2.1.2 Boundary Lubrication

Boundary lubrication is independent of the bulk properties of a fluid. It relies primarily on particular molecules adsorbing to the articulating surfaces, providing a protective layer as small as one molecule thick on the surface. Although ideal boundary lubrication depends on the surfaces involved, several properties have been found beneficial in many metal-on-metal articulations. For example, boundary lubrication requires ordered lining of molecular layers. Therefore, a long, unbranched chain molecule performs better than a shorter molecule or a branched molecule. Furthermore, on metallic surfaces, it is ideal for one end of the molecule to be hydrophobic and the other to be hydrophilic. This configuration facilitates strong chemical adsorption of the hydrophilic end to each metal surface and promotes orderly layer formation. A schematic view of boundary lubrication is shown below in Fig. 2.1.2. These properties, while appropriate for metal-on-metal articulations, may not be ideal for metal-on-PE.

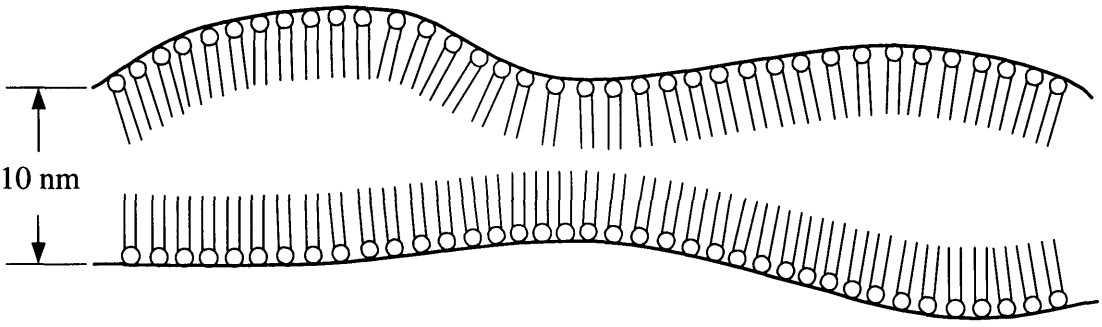

Figure 2.1.2 Schematic representation of boundary lubrication in a metal-on-metal contact The polar end of the molecule binds reversibly to both hydrophilic surfaces. The hydrophobic ends repel, creating an ordered, protective layer one or more molecules thick.

Molecules that fit this description that are commonly used as boundary lubricants include long, unbranched fatty acids and alcohols, although a variety of other molecules can be used. In industrial lubricants, one of these components is typically introduced as a small quantity additive into a grease or viscous oil because small amounts of boundary lubricant are sufficient to coat the articulating surfaces. Good boundary lubricants seek

out the surfaces of the fluid, and so naturally find the articulating surfaces even when present in small concentrations.[13]

After Charnley suggested, in 1959, that the reciprocating motion of synovial joints precluded fluid film lubrication, a number of researchers endeavored to demonstrate that the extremely low coefficient of friction was the work of boundary lubrication. In 1970, Radin and Swann reported a coefficient of friction below 0.005 for cartilage-on-cartilage lubricated by synovial fluid in experiments designed to prevent fluid film lubrication.[38] Such low friction values in the absence of a fluid-film suggested the presence of an excellent boundary lubricant in synovial fluid.

The lubricating advantage provided by this component has been shown to be independent of the bulk properties of the fluid. Several researchers have shown that synovial fluid can provide excellent lubrication to cartilage even after its viscosity has been reduced by degradation using hyaluronidase.[38-41] The component responsible for boundary lubrication of the synovial joint appears to be a protein[42] or one or more phospholipids.[41]

Lubricin

Radin and Swann[38] separated out portions of bovine metatarsal synovial fluid using centrifugation, and used the portions to lubricate a cartilage-on-cartilage articulation under boundary conditions. They found that the best boundary lubricating fraction of synovial fluid did not contain HA (although the "non-lubricating fraction" had a coefficient of friction comparable to that of ice on ice). Since the best lubrication came from a fraction containing mostly protein, they reasoned that a lubricating protein unique to synovial fluid could be isolated.

Swann continued this work using various separation techniques to isolate this lubricating fraction of synovial fluid from bovine metatarsal joints. In 1972, he used electrophoresis to determine amino acid frequencies for this lubricating fraction, which produced a coefficient of friction 30% less than saline.[42] Eventually, Swann was able to isolate from bovine metatarsal synovial fluid a relatively monodisperse glycoprotein of apparent molecular weight 228 kDa that appears to lubricate cartilage under boundary conditions better than other portions of bovine metatarsal synovial fluid.[12,43] He named this protein lubricin.

Jay and coworkers have picked up the work on lubricin where Swann left off, characterizing lubricin[44] and determining its interaction with HA.[45] They later determined that lubricin is made by synovial fibroblasts through the expression of the megakaryocyte stimulating factor gene.[46] Later they found that lubricin can be produced by articular chondrocytes and is homologous to superficial zone protein.[47] Other groups working on lubricin include Caterson *et al.* and Schmidt *et al.* Currently, these researchers are trying to pin down the molecular structure of lubricin and its functional relevance.

Although one group, whose work is discussed below, disagrees altogether with the claim that lubricin is the active boundary lubricant in synovial joints, the mainstream orthopaedic community is beginning to accept lubricin as an important natural joint lubricant. Nonetheless, the role of this protein in the tribology of natural joints is not completely understood.[18,41,48-53]

The role lubricin plays in the boundary lubrication of replacement joints has not been well-studied. It depends on the mechanism of action of lubricin, and its specificity to the synovial joint. If lubricin functions robustly, then it may very relevant to today's replacement joints. If lubricin is unstable, if its production is hampered in TJA, or if it cannot bind appropriately to the surfaces of the implant, lubricin does not likely function in the replacement joint. Once the mechanism of action of lubricin is better understood, prosthesis design may be geared toward preserving the function of lubricin.

Phospholipids

The other major candidate for boundary lubricant in synovial fluid is phospholipid. Phospholipids are the primary lipids in cellular and intracellular membranes, containing a hydrophilic head and two hydrophobic tails. The mechanism proposed for phospholipid lubrication of cartilage is a series of 4.5 nm layers. The first layer is ordered such that hydrophilic heads are attached to articular cartilage, with hydrophobic tails facing outward. Alternate layers are oriented in the opposite fashion. Interlayer interaction by hydrophobic bonds is weak, so that shearing occurs along layer boundaries. Surfaces are separated by gaps on the order of 50 nm.[50]

There is reason to believe that phospholipids provide some boundary lubrication function in the natural joint. Water droplets on fresh articular cartilage create a large contact angle indicative of a hydrophobic surface, in contrast to the highly absorbent (hydrophilic) nature of the bulk material. This hydrophobicity appears to be deficient in diseased areas of arthritic joints.[54] It is supposed by Hills and coworkers that this must be due to a molecule bound to the surface that is hydrophobic on one end and hydrophilic on the other.[49] Phospholipids fit this description[55] and several classes of them, including phosphatidylcholines and sphingomyelins, have been found bound to articular cartilage.[56] Furthermore, the lubrication mechanism of phospholipid bound to articular cartilage is consistent with our current understanding of boundary lubrication of more traditional engineering surfaces, as shown in Fig 2.1.2.

Hills *et al.* are the main proponents of phospholipids as boundary lubricants in the natural joint. They first isolated and quantified the phospholipids in canine synovial fluid in 1984, and used a reciprocating glass-on-carboxylated cotton apparatus to measure friction. Hills showed that μ decreased from 1.5 in dry lubrication to 0.1 using phospholipids isolated from synovial fluid to 0.01 using commercial phosphatidylcholine.[57] These results were difficult to compare to values obtained with natural or replacement joint materials, however, since the articulating surfaces were different. Hills continued to pursue evidence that phospholipids are responsible for boundary lubrication in the natural joint. In 1989, he argued, using scanning electron microscopy, that several layers of stacked phosphatidylcholine on bovine and ovine articular cartilage were responsible for its hydrophobic nature.[55]

In 1998, Hills endeavored to put all controversy to rest, and directly compared the effect of digestion by hyaluronidase, phospholipase A_2, and trypsin on friction between articular cartilage surfaces lubricated by synovial fluid. These enzymes would digest HA, phospholipids, and proteins, respectively. In his experiments, hyaluronidase increased friction by 12% (not statistically significant), phospholipase increased friction by 25%, and trypsin actually *decreased* friction by 30%. From this study, Hills concluded that phospholipids were the boundary lubricant, and were bound by the protein

lubricin. When lubricin was digested, more phospholipids were released to provide increased lubrication, and thus, lower friction.[41]

This result may *seem* definitive, but when Jay repeated the study using latex-on-glass in 1999, he reported that trypsin increased friction.[58] Jay argued that previous studies had been misinterpreted because of some interaction between trypsin and articular cartilage[15] or trypsin and phospholipids[57]. Thus, Jay concluded that lubricin performs the boundary lubrication. The correct interpretation of these findings is still debated in the literature.

Recent work from another group suggests that the loss of lipid from the surface of articular cartilage leads to changes consistent with OA.[59] The authors suggest that lipid depletion disables physiological lubrication, leading to cartilage damage, and eventually OA. This hypothesis is far from proven, however, and the controversy continues over how these constituents contribute to boundary lubrication in the natural joint.

Phospholipid may hold more promise for boundary lubrication in the replacement joint, however. In an extreme pressure wear test using metal ball bearings, synovial fluid and phospholipid extracted from synovial fluid both excelled.[60] Phospholipid has been found to adsorb to PE as well,[61] so it may bind to both surfaces in current replacement joints. Furthermore, adding small amounts (0.5 mg/ml) of phospholipid to a protein-based lubricant reduced wear in a hip simulator by a factor of three.[62] An additional increase in phospholipid concentration further decreased wear. This result suggests that variation in joint fluid phospholipid concentration among TJA patients might account for highly variable wear rates. This result also suggested that variation in phospholipid concentration among bovine serum samples might affect the wear rates found in simulator tests. These issues are discussed in greater detail in a section 2.4.7, but are indicative that phospholipids may play a prominent role in the boundary lubrication of joint replacements.

Other Proteins

There is evidence that other proteins can play a role in synovial boundary lubrication. For example, γ-globulin aided boundary lubrication of swine shoulder joints lubricated by HA and albumin in saline.[63] Both γ-globulin and albumin have been found in substantial quantities in joint fluid from TJA.[64] These proteins, also present in serum, may be relevant in simulator wear tests as well as replacement joints.

Boundary Lubrication in TJA

Boundary lubrication is not well understood in the replacement joint or in laboratory simulations. Any of the components mentioned above may play a role in this lubrication. Of these three, lubricin is the least-well understood, and therefore the most difficult to study. Lubricin has not been manufactured in large quantities; reliable assays for its presence are only now being developed as of the time of this writing. Furthermore, it would be presumptuous to expect that a component important in one articulation (cartilage-on-cartilage) would be of primary import in an articulation with such different surface chemistry and topography (metal-on-PE) without an understanding of its mechanism of action. Finally, wear studies have shown that proteins in joint fluid other than lubricin provide some lubrication of replacement joint materials. For these reasons, the present examination focuses on the role of phospholipid and the most prevalent

proteins in joint fluid as boundary lubricants in the replacement joint. If boundary lubrication by these components is excluded, further study of lubricin may be warranted.

2.1.3 Mechanisms of Lubrication Unique to Synovial Joints

A number of mechanisms have been proposed relating specifically to lubrication of load-bearing synovial joints. These range from fluid film mechanisms involving fluid transfer between cartilage and synovial fluid to electrostatic interactions. These mechanisms are discussed briefly here for completeness, but do not play a substantial role in the thesis as a whole.

One such proposed mechanism, called weeping lubrication,[15] involves the storage of a small amount of synovial fluid in cartilage for release during periods of high loads. This flow provides the energy required to sustain load bearing in the absence of motion. Another possible mechanism, called boosted lubrication is, in a sense, the opposite of weeping lubrication. In boosted lubrication, water is pressed out of synovial fluid and into cartilage under extreme pressure. This leaking increases the viscosity of synovial fluid, and helps support high loads.[18] In one variant of boosted lubrication, the concentrated synovial fluid forms a gel, providing solid lubrication.[65-69] It is also possible that HA-protein complexes form to provide some viscous lubricating advantage.[18] These lubrication mechanisms are of some interest for the lubrication of synovial joints. Nonetheless, since replacement joint materials do not exchange mass with joint fluid in the way cartilage is purported to, these mechanisms of lubrication are not relevant in current arthroplasty. These mechanisms may one day be given consideration in TJA design.

On a smaller scale, electrostatic repulsion has been suggested as a mode of lubrication in natural joints. Roberts and coworkers argued for this type of lubrication.[70] In theory, excess charge contained on the cartilage surfaces repel the opposing surface, thus deforming the very elastic cartilage surface to make it very smooth. This repulsion creates a uniform gap over a larger surface area than would be expected otherwise. He further argued that low ion concentration in the arthritic joint compromised this electrostatic effect, and led to joint stiffness and cartilage wear. He predicted a 10 nm gap between surfaces, which is an order of magnitude less than has been found experimentally in a rabbit model.[9]

In another study, Linn and Radin found that using salt solutions of different pH to lubricate dog ankle joints affected the coefficient of friction. Both high (> 8) and low (< 5) pH were found to reduce friction. They attributed the pH effects to electrostatic adhesion, and the buildup of surface charge leading to repulsion at hydrogen ion concentrations deviating from the isoelectric point.[39]

It is unlikely that charges building up on the surface of implant materials in a prosthetic joint would be sufficient to bear a load at 100 nm. Moreover, none of the materials currently used in joint prostheses are sufficiently elastic or smooth to provide sufficient contact area without surface-surface contact, particularly in the less conforming knee joint. Consequently, electrostatic lubrication is discounted as a relevant mode of lubrication in this thesis.

2.1.4 Lubrication Summary

Flow properties (such as viscosity) characterize the effect of a particular lubricant in fluid film lubrication. In contrast, boundary lubrication depends on the presence of particular components in the fluid capable of adsorbing to the articulating surfaces. Since both fluid film lubrication and boundary lubrication are important in natural joints, it is important to examine both flow properties and composition of joint fluid when considering the replacement joint. The subsequent two sections discuss previous work in the composition and properties of joint fluid.

2.2 Constituents of Synovial Fluid

The two functions of synovial fluid are to lubricate both the synovial membrane and articular cartilage and to nourish the avascular soft tissues of the joint. Nutrients from the blood are filtered through the synovial membrane to reach articular cartilage. Thus, synovial fluid contains small proteins, sugars, ions, and a small amount of phospholipid dialyzed from blood. Other important components of synovial fluid, such as HA are synthesized by Type B synoviocytes. Finally, superficial zone chondrocytes and synoviocytes synthesize proteins, including lubricin, a lubricating protein specific to synovial fluid. Specific proteins and other constituents may be released from chondrocytes in certain disease states. The contribution of these molecules to lubrication in the natural joint has been discussed above.

Any number of these constituents or combinations of these constituents in joint fluid *could* affect the tribology of joint replacement, but few of the components have been examined at all with respect to their role in the lubrication of joint prostheses. Phospholipids and proteins, as discussed above, are two components that have been implicated as boundary lubricants in natural joints; furthermore, both have been studied to a limited extent as lubricants in the replacement joint. HA, a large polymer, greatly affects the flow properties and, therefore, fluid film lubricating ability, of synovial fluid, and has also been shown to interact significantly with both proteins and phospholipids. These components are used as a starting point to examine the tribology of the replacement joint.

2.2.1 The Origin of Synovial Fluid

Synovial fluid resides in a sac between the articular cartilage of the femur and the tibia in the knee, and between the femoral head and acetabular cup in the hip. Synovial fluid is separated from the surrounding tissues by a cellular structure called the synovial membrane. The synovial membrane extends from the edge of the articular cartilage on both bones, and consists of macrophages (Type A synoviocytes) and fibroblasts (Type B synoviocytes).

The synovial membrane does not contain a basement membrane, but is a layer of tissue one to three cells thick[71] that forms a filter between blood and synovial fluid. Its outer surface is highly vascular, covered with fenestrated endothelium. This layer of endothelium prevents large molecules and particles, such as red blood cells and platelets, from entering the joint space from the blood, but allows molecules less than about 10 kDa in size to pass through freely. Larger molecules are filtered to a greater or lesser extent based upon their size. For example, one report gives 7 to 18 mg/ml of albumin (MW ~

66 to 69 kDa) in synovial fluid of healthy patients, but only 0.5 to 2.9 mg/ml of γ-globulin (MW ~ 152 kDa).[71] These components are present in serum in concentrations 35 to 55 mg/ml and 6 to 18 mg/ml, respectively. Still larger proteins, such as fibrinogen (MW ~ 340 kDa) are prohibited entirely from entering the healthy joint. This filtration is one of the primary functions of the synovial membrane.[72]

The cells of the synovial membrane further restrict what components may enter the synovial fluid. Filtration by these cells is not necessarily size-selective, and may include channels for specific molecules such as glucose. Drainage of synovial fluid from the synovial cavity is performed by the lymphatic system. This system does not appear to be selective, and merely drains material from synovial fluid in proportion to its concentration.

Inflammation of the synovial membrane has been found histologically in OA.[73] This suggests a correlation between changes in the synovial membrane and OA, though some contend that histological changes in the synovial membrane are not a primary cause of symptomatic OA.[74] In any case, compromised synovial membrane performance is associated with diseased states. Both decreased and increased permeability have been associated with synovial membrane inflammation.[18] Decreased permeability to sugars can result in starvation of articular chondrocytes, which are nourished by synovial fluid. Chondrocyte starvation leads to an inflammatory response, which can induce increased permeability of the synovial membrane. Increased permeability, on the other hand, can result in clotting due to the admittance of fibrinogen (MW ~ 340 kDa), or, worse, the admittance of inflammatory cells that directly attack the cartilage. Thus, any synovial membrane dysfunction could contribute to impaired tribology and joint degeneration.

Synovial membrane changes are not limited to pathological states, however. For example, experiments in canine knees found that modest exercise increased blood flow to the joint by a factor of three.[72] Blood flow to the joint is controlled by the permeability of the synovial membrane and constriction/dilation in synovial vasculature, though perhaps specific molecular channels could be favored (*i.e.* glucose channels) as opposed to a universally increased permeability. Such changes would clearly affect the composition of joint fluid. It has not been examined in the literature whether such physiological changes affect joint lubrication.

Both synovial vasculature and the synovial membrane can be damaged by the inflammatory process of RA. This disease process differs that observed in OA (*i.e.*, synovial membrane changes without changes in synovial vasculature).[75] This result suggests that differences exist between the composition of joint fluid in OA and RA patients with joint replacement. If the composition of joint fluid affects the lubrication of joint replacements, there is reason to suspect that the underlying disease leading to joint replacement would affect the outcome of joint replacement.

Articular cartilage also contributes to the remarkable properties of the synovial joint. Articular cartilage is one of the major soft tissues nourished by synovial fluid, so it is additionally relevant in determining the composition of joint fluid both from a causal and a teleological standpoint. In the case of joint replacement, however, articular cartilage is completely removed, so it no longer contributes to lubrication. Therefore, it is not appropriate to give a complete review of the structure and function of articular cartilage. Articular cartilage provides substantial lubricating and shock-absorbing

functions to the natural joint that are not imitated in state-of-the-art joint replacements. These differences have been discussed in some detail above in section 1.2.5. Mankin *et al.* give a complete review of the structure and function of articular cartilage.[76]

2.2.2 Protein in Synovial Fluid

Other than water, protein makes up the largest portion of synovial fluid. As such, any consideration of synovial fluid must begin with protein. Furthermore, proteins have been implicated as boundary lubricants in the natural joint, so they deserve consideration as determinants of tribology in the replacement joint. In a healthy synovial joint, most large proteins are filtered by the synovial membrane, leading to a somewhat lower protein concentration in synovial fluid than in serum.

Protein concentration in synovial fluid has been reported for a variety of patient groups. A sample of literature reports are summarized below in Table 2.2.1. Protein concentration in synovial fluid aspirated from the knees of healthy patients has been reported on numerous occasions, and is commonly accepted as close to 20 mg/ml. The reports of Anadere[77] and Rabinowitz[78] and the early report of Balazs[79] demonstrate the wide variety of results in the literature. The later, more comprehensive report of Balazs[80] gives the commonly accepted value. The range of values found cannot entirely be attributed to differences in methodology and laboratory practice, since both standard deviation and the range of values (when reported) are quite high even within a single report. A review by McCarty gives the normal range as 12 to 30 mg/ml.[71]

As shown below, protein concentration in synovial fluid increases almost twice its normal value to about 35 mg/ml in OA.[77,81-83] From the reports shown here, no difference between synovial fluid from the hip and knee is apparent, though it has been reported that protein content is higher in the normal hip than in the normal knee.[72] Wide variability can be noted in most of these reports upon close examination of much of the primary data, though researchers rarely note so. Rheumatoid joints have somewhat higher concentrations of protein in the vicinity of 45 mg/ml,[77,79,81,82,84] and exhibit similarly wide ranges.

A likely source for the discrepancy among patient groups, as well as the variation within individual patient groups, is the integrity of the synovial membrane. As discussed above, the synovial membrane normally filters plasma proteins, so the higher values often encountered in OA and RA may reflect synovial membrane dysfunction. This explanation is favored over variability of plasma protein concentration. One would expect variation of about 25% in synovial fluid protein concentration based upon the variability of plasma protein concentration (normally 60 to 78 mg/ml). Furthermore, major compositional differences have been found between left and right knees, especially among older patients,[85] lending still more support to the hypothesis of local membrane effects, rather than systemic disease, leading to varied composition.

Given the existence of this variability, the question arises whether higher protein concentrations are beneficial or harmful for lubrication of TJA. Recent work by several researchers has examined the related question of how bovine serum protein concentration affects wear in hip simulators. This topic is reviewed below in section 2.4. Chapter 6 of the present work begins to approach more directly the question of how protein contributes to lubrication of TJA.

Table 2.2.1 Total protein concentration in joint fluid from various patient groups Patient groups are described as they were in the original work. Joint fluid samples taken from knees unless otherwise noted. Healthy and normal joints are considered equivalent, as are degenerative and arthritic. Results are presented in mg/ml as mean ± standard deviation, or as a range, as presented in the original work. When necessary, standard error of the mean has been converted to standard deviation. *These patients had damaged menisci, but otherwise normal joints. **Four of these patients had psoriatic arthritis, and 12 had rheumatoid arthritis.

Patients	*Number of Samples*	*Protein*	*Reference*
Healthy	10 pools of ~7	10 – 15	Balazs, 1967[79]
Normal*	7	36 ± 4	Anadere, 1979[77]
Normal	30	29 ± 22	Rabinowitz, 1979[78]
Normal	132	20 ± 5	Balazs, 1982[80]
Degenerative	18	34 ± 6	Anadere, 1979[77]
Degenerative	7	34 ± 4	Punzi, 1986[81]
Degenerative	8	17 – 57	Gomez, 1993[82]
Arthritic Hip	21	32 ± 2	Saari, 1993[83]
Rheumatoid	7	20 – 50	Balazs, 1967[79]
Rheumatoid	11	25 – 57	Swann, 1974[84]
Rheumatoid	34	45 ± 9	Anadere, 1979[77]
Rheumatoid**	12	44 ± 10	Punzi, 1986[81]
Rheumatoid	8	32 – 66	Gomez, 1993[82]
Failed THA	17	34 ± 2	Saari, 1993[83]

Very little has been reported on this topic in the context of TJA. Delecrin *et al.*, using a rabbit model, reported that protein increased for a short time, then returned to normal levels by eight weeks postoperatively.[86] Two authors have reported protein levels after hip arthroplasty. Saari's results, which are included in Table 2.2.1, were taken from failed THAs. Although they may differ from those during successful use of THA, these values were similar to those found in OA before arthroplasty.[83] The other report, from Walker, examined fluid from two cases of THA having 38 and 58 mg/ml protein, respectively.[64] In this report, specific proteins albumin and γ-globulin were measured in both cases. These were present in proportion to their presence in normal synovial fluid as reported previously.[71] These results suggest that, when functional, the synovial membrane filters blood in a similar size-dependent fashion after arthroplasty.

Clearly, there is lacking a definitive study on the concentration of protein in joint fluid in the context of TJA. In particular, only anecdotal work has been reported discussing the protein content of joint fluid after TJA. This gap in the literature is filled in Chapter 4 of this thesis.

Methodology Evaluation – Protein Content

Among the groups listed in Table 2.2.1 who described their methods, protein determination was always performed using the Lowry method. This method, which is a colorimetric assay based upon the binding of copper-protein complexes to a Folin phenol reagent,[87] was once widely used, but has in recent years been replaced by the more robust, sensitive, and rapid Bradford method, which involves measuring the color change of a dimethyl methylene blue dye in the presence of small quantities of protein.[88] The

Bradford method has its limitations as well. First, total absorbance is measured at one wavelength, so does not measure individual proteins. A protein present in small quantities could largely determine tribology, however, especially if it provides boundary lubrication. The potentially significant effects of minor proteins such as lubricin are overlooked by any method that measured only *total* protein concentration. Additionally, since the dye affinity depends on specific amino acids, each protein has a different absorbance. That is, at a given concentration, albumin might absorb more light than γ-globulin does. Consequently, in an unknown solution, this assay provides only a relative measure of total protein (as compared to an albumin standard). It is beyond the scope of this thesis to improve upon the Bradford method.

This methodology raises the question of what individual proteins make up the protein in synovial fluid, and how individual proteins may lubricate replacement joints as well as the effect of total protein content. Work in press by Yao *et al.* suggests that 70% of the protein in joint fluid after TJA is albumin. This determination was made by electrophoretic staining of albumin and four common immunoglobulins. Similar findings (~65% albumin, 12% γ-globulin) were reported by Walker *et al.*[64] In diseased joints, one report gives albumin making up half the protein, and γ-globulin making up another 25%.[89] This is qualitatively similar to the finding in plasma, and is consistent with the expectation that the dominant proteins in joint fluid are small proteins.

Joint Fluid Collection

Before moving on, I will take a moment to discuss issues related to obtaining joint fluid. The means for obtaining joint fluid from human patients varies depending on the patient's circumstances. Synovial fluid is usually most accessible in the knee – orthopaedists regularly remove synovial fluid from knees of patients (arthrocentesis) to relieve pain or to diagnose septic conditions. From patients undergoing open joint surgery (such as TJA), fluid is typically aspirated when the joint is opened. Researchers have, in the past, removed fluid from asymptomatic patients for study, but this practice is less common today due to concerns about risk of infection. Usually between 0.5 and 4 ml can be removed from normal knees by arthrocentesis,[40,85] though it has been widely speculated this does not represent the total amount of fluid in the joint. When the synovial fluid is removed from a joint, osmotic and hydrostatic pressure drives plasma filtrate across the synovial membrane to refill the joint space. Substantially higher volumes of synovial fluid, up to 60 ml,[90] have been found in certain disease states.

One alternative is to examine fluid from animal models, and this has been done in rabbits,[86] dogs,[91,92] and cattle.[93,94] These studies have not been tabulated in the present treatment because human studies are available. Furthermore, preliminary work by others has shown different properties in the joint fluids of humans and cattle,[94] so there is not sufficient reason to expect the composition and properties of joint fluid to be conserved across species. Nonetheless, when human material is not available, animal studies are discussed.

Another alternative is to examine *post mortem* samples,[90,94] Stafford *et al.* showed that synovial fluid becomes diluted after death,[95] perhaps due to hemostatic changes affecting influx across the synovial membrane and efflux through the lymph vessels. Results from *post mortem* studies are presented, therefore, but are not used to

determine the normal range of synovial fluid parameters when other data are available. These considerations are applied to all properties and components of joint fluid.

2.2.3 Hyaluronic Acid in Synovial Fluid

HA is a glycosaminoglycan present abundantly in connective tissue. The repeat unit of the HA polymer consists of alternating *d*-glucuronic acid and *n*-acetyl-*d*-glucosamine (Fig 2.2.1).[96] HA is produced in synovial fluid by fibroblast-like cells called Type B synoviocytes. It is secreted into the joint capsule, where it exists as long, unbranched chains with molecular weight up to 10^7 Da in synovial fluid.[97,98] Though seemingly simple in structure, the HA molecule has some unusual physical properties that have made it the topic of much research.

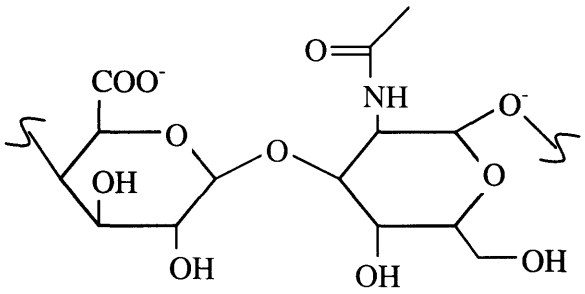

Figure 2.2.1 Schematic of an HA monomer With *d*-glucuronic acid on the left and *n*-acetyl-*d*-glucosamine on the right, the disaccharide monomer of HA is shown.

Since HA is an unbranched polymer, it can be characterized in solution by its molecular weight and concentration. In 1960, Laurent compared light-scattering, centrifuging, and intrinsic viscosity as methods for determining the molecular weight of HA. He concluded that intrinsic viscosity was the best method, and fit his data to the Mark-Honwink equation, reporting

$$[\eta] = 0.036\, M_v^{0.78}, \qquad \textit{Equation 2.2.1}$$

where $[\eta]$ is intrinsic viscosity in ml/g and M_v is the viscosity average molecular weight in Daltons. For this formula, he assumed that the specific volume of HA was 0.66 cm^3/g. Intrinsic viscosity was considered preferable to centrifugation because of deleterious effects of polydispersity on the sensitivity of centrifugation.[99] Since that time, the viscous[100-104] and viscoelastic[105,106] properties of HA have been characterized under a wide range of conditions. These properties depend on shear rate, pH, and solute osmolarity, as well as molecular weight and concentration of HA; the rheological behavior is generally consistent with theoretical predictions for semi-flexible polymer solutions.[107] At physiological molecular weight and concentration, the HA network is a shear-thinning, viscoelastic liquid.[108]

HA is the largest molecule in synovial fluid, and, though less abundant than protein, is thought to contribute more to the flow properties of synovial fluid. Consequently, HA plays a substantial role in determining the efficacy of hydrodynamic lubrication by synovial fluid in the natural joint. Since HA also exists in joint fluid after TJA, it may contribute in this manner to the fluid film lubrication of the replacement joint. Furthermore, the entanglement network HA chains form at physiological molecular weight and concentration[12] may affect the tribology of TJA in a more complex manner, potentially involving its interaction with other molecules.

HA may serve many functions in the synovial joint. For example, it has been hypothesized that an entangled HA network can slow the diffusion of cytokines through synovial fluid, reducing scar tissue formation, inflammation, and, in a replacement joint, foreign body response. Indeed, HA has been found to suppress the formation of granulation tissue around foreign polyethylene bodies, and to improve wound healing in animal models.[80] Although there is some debate as to *how* HA protects the joint, its crucial role in joint protection is demonstrated by the fact that digesting HA by injecting hyaluronidase into the knee induces OA.[109]

Hyaluronic Acid Concentration in Synovial Fluid

A number of groups have reported on the concentration of HA for various groups of patients. A selection of these studies is summarized below in Table 2.2.2. The work of Balazs,[79,80] using more reliable methods than earlier work, established a range of 1 to 4 mg/ml as the normal concentration of HA in synovial fluid. In OA knees, several authors found mean concentration close to 1 mg/ml,[77,90,95,110] though the range, as reported by Gomez *et al.*,[82] was quite large. In rheumatoid arthritis, an equivalently large range of concentration has been found,[82] but in cases in which an author reported HA concentration in both RA and OA fluids, RA fluids had less HA. The quantity of HA in joint fluid from hip replacements has been examined a few times, and appears to be less than that in OA hips.[83,110]

HA has not yet been measured from joint fluid of human subjects with TKA, but HA most likely still exists in the joint after arthroplasty (it has been shown in THA).[64,83,110] This finding supports the work of Namba *et al.*, who found that fibroblasts lining the pseudocapsule, like type B synoviocytes in the synovial membrane, produce HA in arthroplasty patients.[111] Whether their production of HA matches that prior to arthroplasty is an issue that is answered by a comprehensive examination of HA concentration and molecular weight before and after TJA (see Chapter 4)). Evidence in a rabbit model, however, suggests that HA exists in different concentrations in joint fluids of natural and prosthetic joints,[86] though the natural joints used for comparison were not diseased.

Since HA is produced by Type B (fibroblast-like) synoviocytes in the synovial membrane, the amount of HA in the joint is controlled by the number and function of these cells. The concentration of HA in joint fluid is also dependent on the amount of fluid in the joint, and thus proper function of the synovial membrane and lymph system. From this standpoint, synovial membrane inflammation and permeability changes associated with both OA and RA[18] may be sufficient to explain the decreased and variable concentration of HA in these disease states.

It has been shown that concentration of HA affects the bulk properties of synovial fluid.[112] The properties include flow properties such as viscosity, but also include important chemical properties such as the diffusivity of various molecules. The diffusivity of a particular cytokine in synovial fluid could be important in determining, for example, the strength of an inflammatory response to some insult on articular chondrocytes. Since this is the case, it is not clear whether the decrease in HA concentration is a cause or a result of the arthritic condition.

Table 2.2.2 HA concentration in joint fluid from various patient groups Patient groups are described as they were in the original work. Joint fluid samples taken from knees unless otherwise noted. Healthy and normal joints are considered equivalent, as are degenerative and OA. Methods used are discussed in the text. All data are presented in mg/ml as mean ± standard deviation or as a range, as presented in the original work. When necessary, standard error of the mean has been converted to standard deviation. *These patients had damaged menisci, but otherwise normal joints.

Patients	*Number of Samples*	*Hyaluronic Acid*	*Reference*
"Normal"	8	4.1 ± 1.0	Stafford, 1964[95]
Healthy	10 pools of ~7	1.4 – 2.9	Balazs, 1967[79]
Healthy	132	3.2 ± 0.6	Balazs, 1982[80]
Normal*	7	1.0 ± 0.4	Anadere, 1979[77]
Post mortem	13	2.4 ± 0.9	Stafford, 1964[95]
OA effusion	19	1.2 ± 0.7	Stafford, 1964[95]
Degenerative	18	0.9 ± 0.2	Anadere, 1979[77]
OA	1	0.9	Dahl, 1985[90]
Degenerative	8	0.3 – 3.6	Gomez, 1993[82]
OA	23	1.1 ± 0.3	Yamada, 2000[110]
OA Hip	21	2.2 ± 0.2	Saari, 1993[83]
RA effusion	22	0.7 ± 0.3	Stafford, 1964[95]
Rheumatoid	7	0.4 – 1.9	Balazs, 1967[79]
Rheumatoid	13	0.8 – 1.7	Swann, 1974[84]
Rheumatoid	34	0.5 ± 0.2	Anadere, 1979[77]
Rheumatoid	10	0.5 – 1.3	Dahl, 1985[90]
Rheumatoid	8	0.7 – 3.7	Gomez, 1993[82]
THA	3	0.28 – 0.56	Walker, 1973[64]
Failed THA	17	0.43 ± 0.04	Saari, 1993[83]
Loose THA	13	0.6 ± 0.4	Yamada, 2000[110]

There may be other causes of this variability, however. For example, most studies did not consider what pharmacological interventions may have affected the environment of the joint. Dahl *et al.* found that the concentration of HA is not affected by the administration of non-steroidal anti-inflammatory drugs,[90] though other treatments may have an affect. In particular, viscosupplementation is a pharmacological intervention which may effect the HA composition of joint fluid. Finally, given the wide range of causes of OA, it seems likely that different disease etiologies could give rise to different synovial fluid compositions.

Methodology Evaluation – Hyaluronic Acid Concentration

In 1953, Ogston reported that placing synovial fluid in a low pH environment makes HA bind with protein, forming a mucin clot.[93] A decade later, by weighing mucin clots, Stafford estimated the concentration of HA in the synovial fluid of healthy and arthritic knees.[95] A limitation of this method is that it relies on proteins in the fluid, which can vary, to determine HA in the fluid. Consequently, the 4 mg/ml reported by Stafford is likely less accurate than later reports.

A few years later, Balazs used hyaluronidase to digest HA[93] and then measured the concentration of hexosamine and hexuronic acid in the resultant solution to estimate

HA concentration.[79] This method is considered more reliable than the previous method using the mucin clot, so Balazs's range is considered representative. Other methods, including carbazole reaction[64,82] (a.k.a. the Dische method[113] and centrifugation[84] have been used, and have yielded results similar to Balazs's. Kongtawelert *et al.* have developed a number of methods to determine HA concentration using ELISA-like assays.[114,115] These methods depend on obtaining rare monoclonal antibodies, and thus have not been extensively used. Presently, the carbazole reaction is often used, and is employed in Chapter 4 of this thesis.

Hyaluronic Acid Molecular Weight in Synovial Fluid

The molecular weight of a polymer follows a distribution, but can be characterized by one or a few parameters. There are at least four distinct measures of molecular weight, and they differ based upon the polydispersity of the sample. M_v, the *viscosity* average molecular weight, is determined using the calculation of the Mark-Honwink Equation (*i.e.*, Equation 2.2.1). This can only be determined for pure samples, such as HA in solution. M_n, the *number* average molecular weight, is calculated by summing the number of molecules at each molecular weight over all molecular weights, and dividing by the total number of HA molecules. M_w, the *mass* average molecular weight, is calculated by summing the mass at each molecular weight over all molecular weights and dividing by the total mass of HA in the sample. M_z, the *z*-average molecular weight, is calculated by summing the square of the mass at each molecular weight over all molecular weights, and dividing my the square of the total mass of HA in the sample. Thus, M_z and M_w weight large molecules over small molecules, whereas M_n considers all particles equally. As a rule, $M_n < M_v < M_w < M_z$. The ratio of M_w to M_n reflects the polydispersity of a polymer, with $M_w = M_n$ indicating a single molecular weight. Finally, M_p, the *peak* average molecular weight, is the molecular weight corresponding to the elution time of the peak of a species through a chromatography column. M_p can be thought of as the mass mode of the sample.

The dissemination of a "normal" HA concentration and molecular weight[116] in human joints belies the lack of a definitive work on the subject. Unlike protein and HA concentration, no simple method has emerged to determine HA molecular weight in joint fluid. Although there have been several studies on human joint fluid, the methodology has been highly varied. Based upon limited studies in arthritic patients and THA patients, the molecular weight range of HA may be slightly lower in both groups than in normal joints, but is of the same order of magnitude and appears to be as polydisperse.[83,90,110] The limited data allow little comparison to be made between groups, but what differences do exist may be explained by differences in synthesis, stable chain length, or degradation of HA in various disease states. The methodology of each of the reports summarized in Table 2.2.3 is discussed in more detail below.

Table 2.2.3 HA molecular weight in joint fluid from various patient groups Patient groups are described as they were in the original work. Joint fluid samples taken from knees unless otherwise noted. Arthrotic and OA joints are considered equivalent. Methods used are discussed in the text. Data are presented in millions of Daltons as mean ± standard deviation or as a range, as presented in the original work.

Patients	*Number of Samples*	*Molecular Weight*	*Reference*
Normal	2	M_p 6.0 – 6.8	Lee, 1994[117]
Post mortem	5	M_n 1.9 ± 0.8 M_w 7.0 ± 0.5	Dahl, 1985[90]
OA	1	M_n 0.3, M_w 4.2	Dahl, 1985[90]
Arthrotic	5	M_w 2.8 ± 0.2	Kvam, 1993[118]
OA	23	M_v 3.8 ± 2.0	Yamada, 2000[110]
OA Hip	21	1.1 ± 0.8	Saari, 1993[83]
RA	12	M_n 0.8 ± 0.4 M_w 4.1 ± 1.4	Bjelle, 1982[97]
Rheumatoid	10	M_n 0.6 ± 0.5 M_w 4.8 ± 1.1	Dahl, 1985[90]
Failed THA	17	2.6 ± 1.1	Saari, 1993[83]
Loose THA	13	M_v 3.1 ± 0.9	Yamada, 2000[110]

Methodology Evaluation – Hyaluronic Acid Molecular Weight

When dealing with pure samples, intrinsic viscosity is the preferred method for molecular weight determination of polymers. This method can be used as a stand alone method (to determine M_v) or in conjunction with a separation column (as in size exclusion chromatography (SEC), to determine molecular weight distribution). Unfortunately, the stand alone method cannot be appropriately applied to complex solutions such as joint fluid due to the possibility of intermolecular interactions (*e.g.*, between protein and HA). Analytical centrifugation is another method that has been used, but it requires the specific gravity of HA, which varies depending on the solvent.[98] Therefore, it cannot be easily applied to joint fluid samples. In short, due to the complex nature of joint fluid, it is difficult to apply traditional analytical chemistry to the measurement of HA molecular weight in joint fluid.

In 1964, Stafford approached an HA molecular weight estimate by measuring the intrinsic viscosity of joint fluid samples.[95] He rightly did not try to apply Equation 2.2.1 to the data, since Laurent's equation was derived for pure HA only. In 2000, Yamada *et al.* did apply this equation to estimate the molecular weight of HA in OA and THA.[110] This method might be reliable if HA completely determined the viscous properties of joint fluid. Oates *et al.* have shown, however, an interaction between proteins and HA that increases the viscosity of the solution over HA alone.[119] More directly, Swann showed that synovial fluid intrinsic viscosity overestimates HA molecular weight due to intermolecular interactions not present in pure HA solution.[12] Not surprisingly, the molecular weights obtained in THA by Yamada *et al.* were larger than one might expect given the other measures of molecular weight obtained by others.

In 1982, Bjelle *et al.* reported the use of a chromatography column to separate HA by molecular weight, employing an ultraviolet light detector at 206 nm to measure the

quantity eluting from the column.[97] Similar data using similar methods were reported in 1989 and 1993 by Saari *et al.*[83,120] Using an appropriate combination of column and buffer, both groups report being able to separate HA from the proteins in the sample, and thus calculate HA molecular weight. This method has limitations, however. First, it does not confirm the identity of the eluent. Second, although Bjelle *et al.* used density gradients to separate HA from other molecules, Saari *et al.* did not try to prevent protein-HA interactions from affecting elution time. Furthermore, it is difficult to isolate HA using this method, since proteins absorb more at 206 nm than HA does. Consequently, they may have overestimated molecular weight of HA, as reflected in Table 2.2.3. If sufficient separation can be attained between the species, however, the method can be useful. In Chapter 4, an attempt is made to use the methods of Saari *et al.* to determine HA molecular weight in the context of TJA.

In 1985, Dahl *et al.* combined a radioassay technique described by Laurent and Tengblad with SEC to measure HA concentration and molecular weight in synovial fluid from patients with rheumatoid arthritis and other joint diseases.[90] They fractionated the fluid by elution time, which relates inversely to the logarithm of molecular weight, and then performed the radioassay on each eluting fraction. Graphing HA concentration by residence time provided an estimate of molecular weight distribution; adding up the total amount of HA eluted gave the concentration. Laurent reported a 12% standard error in concentration measurement using this radioassay method.[99]

One advantage of this method over other SEC methods is that it confirms the content of HA by means other than molecular weight, so some other high molecular weight component would not be mistaken for HA. On the flip side, it is likely that interactions with proteins affect the apparent size of HA in SEC, and Dahl's method neither guards against nor measures this affect. Another criticism of this method is that Dahl may not have diluted his samples sufficiently to prevent intermolecular interactions from affecting elution time. His results exhibited a distribution characteristic of the sequential elution of boluses of solute, rather than the typical curve of polymer polydispersity.

These limitations were overcome by Kvam *et al.*, who used a purification protocol to eliminate protein-HA interactions before using SEC to determine HA molecular weight.[118] The purification protocol he used also diluted HA substantially (though perhaps incidentally), thus reducing the molecular interactions affecting elution time. The results of Kvam show a molecular weight distribution typical of a disperse polymer. I consider this to be the most reliable of the reports in Table 2.2.3, though it represents a small sample size.

More recently, Lee *et al.* used electrophoresis to determine the molecular weight of HA in joint fluid.[117] Although they were able to determine a peak of HA in synovial fluid, they refused to try to calculate M_n or M_w because no correlation between HA mobility and molecular weight had been determined at molecular weights above 6 MDa. Furthermore, the qualitative nature of electrophoresis takes some of the strength away from any molecular weight calculations based upon band intensity and thickness. Neither this criticism nor Lee's rejection of the method has prevented others from reporting HA molecular weight in synovial fluid using this method, however.[91]

Due to the limitations in each of these approaches, it is difficult to ascertain whether there are real differences between the experimental groups previously studied. The causes of any real difference between these groups may be the same as those causing concentration differences, which are discussed above. Notably absent from the literature is a comprehensive study of HA molecular weight in joint fluid of patients with OA and patients with TJA.

Size Exclusion Chromatography

Of the methods which have been used to estimate molecular weight of HA in synovial fluid, SEC is the most widely used. SEC consists of two parts: a column, comprising a means to separate large molecules from small molecules by retention time; and a detector, comprising a means to record the particles as they elute. Common detectors include ultraviolet and visible light, intrinsic viscosity, refractive index, and light scattering. Even though light scattering (*e.g.*, Seikagaku, Kabi-Pharmacia)[121] and intrinsic viscosity (*e.g.*, Hylauron,[122] Life Sciences) are used to calculate the molecular weight of commercially-available HA, some HA manufacturers argue that these detectors assume a molecular shape that HA does not employ.[122] Another detector option, refractive index increment, has an error of up to 17%. Since its square is used in calculating molecular weight, the error in this method can be quite high.[98] Furthermore, sample impurities invalidate both refractive index and light scattering measurements. Ultraviolet and visible light absorption present their own challenges, since HA has an unimpressive absorption spectrum compared with many of the proteins present in some joint fluid samples. Refractive index, despite its high variability, has been used most commonly to determine HA molecular weight. In Chapter 4, both ultraviolet absorption and refractive index are used to record HA elution.

There are a number of challenges associated with finding an appropriate column. At low molecular weight, HA content has been evaluated by SEC for some time,[123] but, as recently as 1998, no column had demonstrated the ability to separate molecules larger than 1 MDa.[98] More recently columns have become available to do this. These columns contain channels with pores of various sizes, designed to extend the distance smaller molecules travel (and thus, their residence time) before eluting. Entrance into these pores is based upon molecular radius, so the column might not separate a stiff, linear polymer in the same manner as a coiled molecule. Since the shape of HA in solution is not well understood,[98] it is not clear analytically how a column will perform in separating HA of different molecular weights. Moreover, it has been difficult to calibrate these columns, since the molecular weight HA standards must be confirmed by some other means (such as intrinsic viscosity). Typically, the approximation that M_v is equivalent to M_p or M_w must be used to generate a standard curve by SEC.

Since molecules in joint fluid interact in a complex and incompletely understood manner, it is appropriate to minimize their interaction prior to use of size exclusion. In particular, Kvam *et al.*[118] used proteolytic degradation to reduce the interaction between protein and HA. They showed that apparent HA molecular weight was reduced by eliminating this interaction, suggesting that protein-HA complexes increased the apparent molecular weight found by other authors using SEC.

Effect of Storing Joint Fluid on Hyaluronic Acid

Before departing from this section, I will make a few brief comments about handling of joint fluid and its effects on HA. Stafford found that refrigerating synovial fluid for a year did not affect its intrinsic viscosity[95]. This suggests stability in both HA and associated proteins, and suggests that long-term refrigeration can be appropriate storage for joint fluids. Another author has suggested that HA degrades slightly due to the process of freezing and thawing,[121] though this has been disputed.[124] Consequently, appropriate storage of joint fluid over the short term is refrigeration. Over the long term, deep freezing is appropriate, but freeze-thaw cycles should be minimized.

Viscosupplementation

A related topic of note is viscosupplementation, a pharmacological intervention in which a series of injections of HA or its sodium salt are made into arthritic joints to delay the effects of arthritis. The impetus behind viscosupplementation was that injecting high molecular weight HA into the joint may restore the normal rheological properties of synovial fluid, thus promoting normal synovial lubrication.[125,126]

Others have suggested alternate modes of functionality to viscosupplementation. For example, HA injections may decrease the permeability of synovial fluid, thus slowing the movement of inflammatory regulators from the site of cartilage damage. The reduced motility of regulators could inhibit inflammatory response, and therefore reduce subsequent cartilage damage. Other arguments have been put forth to explain the possible benefits of intra-articular injection of HA[52] as well. For a complete outline, the interested reader should consult one of the many reviews on the topic.[116,126-135]

Typical viscosupplements are administered in a series of intra-articular injections over the course of several weeks. Supartz (Seikagaku, Tokyo), one HA supplement, comes in 10 mg/ml concentration at advertised molecular weight between 0.62 and 1.17 MDa. Its pH is between 6.8 and 7.8, and it reports an intrinsic viscosity of 11.8 to 19.5 dl/mg. Orthovisc (Anika Therapeutics, Waltham, MA), another HA supplement, comes in 13.6 mg/ml with listed average molecular weight of 1.39 MDa. Its ionic strength is reported at 316 milliosmoles, and its pH is reported at 5.9. The rheological properties of these joint supplements were reported as part of my master's thesis.[136]

There are conflicting data regarding the function of viscosupplementation from both *in vitro* studies and clinical trials. For example, Mensitieri *et al.*[124] and Smith *et al.*[91] give conflicting reports as to whether viscosupplementation can stimulate endogenous production of HA, or whether it merely contributes its own mechanical properties during its residence time in the joint. HA has an estimated half life of 12 to 24 hours in the joint cavity, based upon animal studies.[116,137]

In a rabbit model, Sonoda *et al.* found biochemical evidence, but not gross morphological evidence, that HA injection provided increased collagen remodeling of damaged meniscus over the course of twelve weeks.[138]. The quantitative differences between treated and untreated joints were not statistically significant. In 2000, Kobayashi *et al.* found, based upon histological examination of collagen degradation, that HA injection provided some protection to damaged meniscus over the course of six months in a rabbit model. Again, the differences were not statistically significant, so the authors were merely able to *suggest* that joint supplementation brought about protection.[139] Others have found histological evidence of increased healing after

meniscus injury with HA injection, but they could not confirm their results biochemically.[140] A number of different injury models have been studied, with similarly promising, but not definitive, results (*e.g.*, Sonoda *et al.* 2000,[141]), though others have found definitive histological effects of viscosupplementation.[142-146]

There are also mixed reviews as to whether viscosupplementation succeeds clinically. A main confounding factor is the impressive placebo effect of saline injection, which might cause one to wonder if the placebo itself provides real benefit. Original clinical studies and reviews are continually being published demonstrating the efficacy or inefficacy above placebo of this treatment.[147-152] A common conclusion regarding the mixed reviews of such studies is that HA injection is a non-steroidal, non-surgical therapeutic alternative for patients in whom other pharmacological interventions are unsuccessful.[153]

Viscosupplementation is relevant to joint replacement because it provides a possible commercial endpoint for research on lubrication of TJA. Given a better understanding of the tribology of TJA, it is reasonable to imagine a biocompatible lubricant (or joint fluid supplement) for the replacement joint that reduces the generation of wear particles.

2.2.4 Phospholipid in Synovial Fluid

Phospholipid is a third candidate for lubricant in TJA, since they have been implicated in boundary lubrication of synovial joints. In particular, cholinated phospholipids such as L-α-dipalmitoyl phosphatidylcholine (DPPC), also called surface active phospholipids (SAPL), may provide some boundary lubrication in natural or replacement joints. SAPLs make up 45% of the phospholipid in normal synovial fluid,[57,154] and 15% of the total lipids in normal synovial fluid. These phospholipids are presumably dialyzed from blood serum, where they are present in 1.5-3.8 mg/ml in healthy individuals.

Since there has been relatively less consideration given to the effect of phospholipid on synovial joints, few reports exist of its concentration in synovial fluid. These results are summarized below in Table 2.2.4. The "normal" phospholipid content is often reported at 0.13 to 0.15 mg/ml, though there are very few measurements reported in the literature. The simplest and most reliable methods have determined phospholipid concentration in OA at 0.3 ± 0.1 mg/ml, though a more comprehensive study is desirable. The value in RA, by all accounts, is much higher, at 0.6 to 0.8 mg/ml. Though the reason for this difference is unresolved, it is likely related to the pathophysiology of the disease. Cholesterol and lipoproteins have also been shown to be present in larger quantities in RA as compared to OA.[89,155]

In 1962, Bole measured a mean phospholipid concentration of 0.14 mg/ml in three pooled samples of normal synovial fluid.[156] It had been previously noted, but not quantified, that phospholipid existed in small quantities in synovial fluid from healthy patients. The protein and HA content he reported were consistent with normal values later reported by others. In RA, he found a much higher mean of 0.84 mg/ml among 24 patients, and quite a wide range. He found a positive correlation between protein concentration and phospholipid concentration, and found no effect of steroid treatment on phospholipid content.

Table 2.2.4 Phospholipid concentration in joint fluid from various patient groups Patient groups are described as they were in the original work. Joint fluid samples taken from knees unless otherwise noted. Degenerative and OA joints are considered equivalent. Results are presented in mg/ml as mean ± standard deviation, or as median (range), as presented in the original work. When necessary, standard error of the mean has been converted to standard deviation. *Four patients had psoriatic arthritis and 12 had RA.

Patients	*Number of Samples*	*Phospholipids*	*Reference*
Normal	3 pools	0.13 – 0.15	Bole, 1962[156]
Normal	30	0.65 ± 1.60	Rabinowitz, 1979[78]
Degenerative	5	0.85 – 1.14	Chung, 1962[157]
Degenerative	7	0.69 ± 0.12	Punzi, 1986[81]
OA	10	0.28 ± 0.09	Prete, 1997[158]
Rheumatoid	10	0.77 – 1.17	Chung, 1962[157]
Rheumatoid	24	0.84 (0.2 – 1.4)	Bole, 1962[156]
Rheumatoid*	16	0.89 ± 0.21	Punzi, 1986[81]
Rheumatoid	10	0.60 ± 0.08	Prete, 1997[158]

In 1979, Rabinowitz *et al.*,[78] using a much larger cohort, found an average phospholipid concentration to be 0.65 mg/ml, but with quite a wide range. They reported protein levels in this group 50% higher than others had reported from normal subjects, raising suspicion of how "normal" the synovial fluid samples really were. In a later publication, they discounted some of their samples, increasing the mean to 0.78 mg/ml.[154] Perhaps due to the uncorroborated protein values, or due to the wide spread in data, this group's findings are not commonly cited in the literature. Interestingly, there have been very few studies of phospholipid in normal synovial fluid since Rabinowitz *et al.*, and Bole's mean of three samples is repeatedly reported as the "normal" value.[159-161] Although there has been little confirmation of these original values from normal human subjects, similar values have been reported in a porcine model.[63]

In 1962, using chromatographic methods, Chung found phospholipid concentration close to 1 mg/ml in both OA and RA.[157] In 1986, Punzi *et al.* reported phospholipid content from a small group of patients with RA or OA. They reported their results in micromoles per ml; the values reported in Table 2.2.4 are based on an assumed molecular weight of 740 Da.[81] More recently, using spectrophotometry, Prete *et al.* evaluated 10 OA and 10 RA patients, finding roughly twice the amount of phospholipid in RA than in OA, and that twice still the normal value.[158] The causes for the difference between normal, OA, and RA synovial fluid are likely similar to those that give rise to the difference in protein concentration, as discussed in section 2.2.2.

Phospholipid concentration in joint fluid has not been examined, to my knowledge, in the context of TJA. Whether the factors which bring about this change in concentration affect joint fluid in TJA, and how this impacts the tribology of TJA are addressed in Chapters 4 and 6.

Methodology Evaluation – Phospholipid Concentration

The first methods used to measure phospholipid concentration employed thin layer chromatography, involving many time-consuming or labor-intensive steps. These methods resulted in the wide variety of results found. More recent work employs a relatively simple colorimetric assay involving the release of choline by hydrolysis of

phospholipids using phospholipase D.[158] Currently, an assay based on the same concept has become commercially available. This assay is specific and sensitive for phospholipids, and is easy to perform.[162] This assay was first used to measure small amounts of phospholipids in serum, but has been adapted to measure them in joint fluid for Chapter 4 of this thesis.

2.2.5 Interactions among Major Components of Synovial Fluid

The contribution of each of these components to TJA lubrication cannot be considered independently. Interactions have been found between these components such that each must be considered in light of the presence of other components. An interaction would be expected between HA and proteins, since semi-flexible polyelectrolytes form complexes with charged spherical molecules like proteins.[163] Specifically examining HA, certain serum-derived proteins have been found to bind to HA.[164,165] Furthermore, albumin has been shown to complex with poly(ethylene glycol),[166] a much simpler polymer than HA. Others have shown that aggrecan can bind to HA, thereby increasing the effective viscosity of the solution.[167] There is also evidence of the formation of thin membranous sheets between HA of high molecular weight and DPPC, a phospholipid.[168,169] Finally, a repeating hydrophobic patch on HA subunits has been shown to be a binding site for phospholipids.[169,170]

These interactions likely affect the rheological properties of joint fluid, thus affecting fluid film lubrication. Old rheological studies suggested a relationship between apparent viscosity and the concentration of an HA/protein complex.[171,172] In a much more recent study, Jay *et al.* reported that proteins added to HA solution could *either* increase *or* decrease the viscosity at high shear rates.[45] Oates *et al.* found that adding proteins to HA solution increased viscosity at low shear rates.[119] These reports are all consistent with the disparity between synovial fluid viscosity and the viscosity of HA solutions alone.

The effects of these complexes are likely not limited to the rheology of joint fluid, however. Complexes with HA may protect DPPC from digestion by phospholipase A_2, enabling the molecule to function more effectively as a boundary lubricant. One group has proposed that interactions among cholesterols may enable lubrication by liquid crystals in the synovial joint.[173] Additionally, they may affect means of determining HA molecular weight in synovial fluid.[174] The importance of these and other interactions are not well-understood with regard to the lubrication of replacement joint prostheses.

2.2.6 Other Components of Synovial Fluid

There are a number of components of joint fluid other than proteins, phospholipids, and HA that receive some mention in arthritis literature. Most are serum-derived, but some are produced by articular cartilage. These components are here considered in light of their potential contribution to replacement joint lubrication.

Arthritis Markers

A number of molecules are present in synovial fluid in small quantities in arthritis. These molecules have been studied as markers for disease severity. As an example, keratan sulfate (normally present in articular cartilage) can be released into synovial fluid in OA. A correlation has been shown between the concentration of certain

components of keratan sulfate in synovial fluid and stage of disease.[175,176] Likewise, chondroitin sulfate isomers can be used as markers of osteoarthritis in synovial fluid. Other markers have been identified as indicative of abnormal proteoglycan synthesis and metabolism in articular cartilage.[177-179] Several markers, either in serum or in synovial fluid, aid in distinguishing OA from RA, predicting disease progression, and monitoring therapies.[180]

These markers exist in synovial fluid in picogram and nanogram amounts,[181] and would not be expected in joint fluid of patients with TJA because their source (*i.e.*, articular cartilage) has been removed.[182] There is no reason to believe that small amounts of these molecules contribute substantially to lubrication in joint arthroplasty. One long-term goal of this research is to establish the components in joint fluid which determine the tribology of TJA. Having determined the role of these components, it may be possible to assay for them in the joint fluid of TJA patients, perhaps even pre-operatively, to better predict patient outcome. Related notions are presently in practice, as in the use of a serum assay in which HA and interleukin-1β are considered markers for aseptic prosthesis loosening in THA.[183]

Glucose and Ionic Strength

Articular cartilage metabolizes a small amount of glucose in the natural joint. There is an active transport mechanism for glucose across the synovial membrane into the joint space. Outward diffusion and lymphatic drainage limit the glucose concentration in the joint in the absence of normal metabolism. In sepsis, glucose levels are low because of increased metabolism by an infectious agent. In inflammatory disease, concentrations may be low due to inhibited transport across the synovial membrane.[72] In the replacement joint, metabolism of glucose in the joint capsule is decreased, so the concentration in the joint capsule is mediated by diffusion and active transport. Although glucose content may vary in TJA, there is no evidence for its importance in tribology.

Although no normal range has been established for the osmolality of joint fluid, it has been suggested that it is similar to serum osmolality.[72] Ionic transport likely follows a diffusive pattern across the synovial membrane. It is important that any simulation of joint fluid mimic physiological osmolality because the rheological properties of HA (and therefore possibly joint fluid) depend on ionic strength.[105] Although buffered saline is often used to simulate the osmolality of joint fluid in certain laboratory experiments,[184] the ASTM standard for joint replacement wear tests does not make such a specification.[185]

Dissolved Gases and pH

The dissolved oxygen and carbon dioxide content of synovial fluid depend on the performance of the synovial membrane and the metabolic demands of the joint's articular cartilage. The concentrations of these gases determine the pH of synovial fluid. Given normal diffusion of oxygen and carbon dioxide, one would expect a pH of 7.35 to 7.45 in the natural joint, as in serum. In 1966, Cummings *et al.*, using electrodes attached to a small needle, measured the pH of synovial fluid intra-articularly, and found a normal range of 7.3 to 7.6 from seven knees. They found that seven of eight RA patients had a pH of 7.1 to 7.3 (one was 7.5), and one OA patient was tested, having a pH of 7.5.[186] Much more recently, Kitano *et al.* measured the pH of synovial fluid extra-articularly in

OA and RA, finding a mean pH of 7.9 in OA (range 7.4 to 8.1), 7.5 in RA (range 7.4 to 7.6), and 8.1 in patients undergoing arthroplasty (range 7.5 to 8.5).[187] These higher values, which have been confirmed by others using similar extra-articular methods[8] may have been affected by oxygenation after fluid extraction.

In the natural joint, high or low pH could damage articular cartilage.[72] In the replacement joint, this is not an issue, but pH can still importantly affect the rheological properties of joint fluid and the binding affinity of various molecules. The rheology of HA has a strong pH-dependence.[105] Furthermore, it has been noted that the interaction between phospholipids and HA is pH-dependent.[186] The behavior of dominant proteins albumin and γ-globulin may change as the pH crosses their isoelectric points. Thus, pH may have an effect on the lubrication of TJA.

2.2.7 Summary of Components of Joint Fluid

Preliminary reports suggest that the major components of joint fluid are the same as those in synovial fluid: protein, HA, and phospholipids. The amount of these components in healthy, OA, and RA patients, as well as available arthroplasty data, are summarized below in Table 2.2.5. There is reason to believe that each of these contributes to either fluid film or boundary lubrication in TJA, though the quantities and relative importance of these components has not been established. Simple, reliable methods have been established for the determination of protein, HA, and phospholipid concentration, but the best method for measuring HA molecular weight has not been established.

Table 2.2.5 Previous work in components of joint fluid This table captures the typical range of parameters for healthy and diseased synovial fluid and joint fluid that will be used in this thesis. When values are well-established, they are given by a range including 95% of all samples (representing two standard deviations of the mean if normally distributed). When conflicting values have been reported, a weighted average of ranges is given. When values are less well-established, they are estimated from available data, and designated with a tilde (~). References and explanations are given above in the text.

	Healthy	*OA*	*RA*	*TJA*
Protein	10 – 30 mg/ml	24 – 44 mg/ml	27 – 63 mg/ml	~ 35 mg/ml
Hyaluronic Acid	~ 2 MDa 2 – 4 mg/ml	M_w 2.4 – 3.2 MDa 0.5 – 1 mg/ml	M_n ~ 0.6 MDa 0.1 – 0.9 mg/ml	Unknown ~ 0.5 mg/ml
Phospholipids	~ 0.1 mg/ml	0.1 – 0.5 mg/ml	0.4 – 0.8 mg/ml	Unknown

2.3 Rheological Properties of Synovial Fluid

All modes of fluid film lubrication in the synovial joint depend on the flow properties of synovial fluid. Most commonly, the flow properties studied in conjunction with fluid film lubrication are viscous and viscoelastic properties. In steady shear flow (*e.g.*, Couette flow), the steady shear viscosity, η, is the fluid property that determines the gap required to bear a given load. The relationship between flow properties and surface gaps is less simple in more complex flow patterns, like the rolling, sliding, and oscillatory motions that exist in human hip and knee articulations. In these situations, viscoelastic properties[80] and extensional viscosity[188] play a role in maintaining fluid-film conditions. Viscoelastic properties may be particularly important in small amplitude oscillatory motions, such as those occurring during running and jumping.

2.3.1 Steady-Shear Viscosity

Consider the case of two parallel plates separated by a fixed gap, h, which is filled with fluid (see Fig. 2.3.1 below). If the bottom plate is held rigidly in place, and a horizontal force is applied to the top plate, the top plate moves, and eventually reaches a steady velocity, V_0. The final velocity depends on the geometry of the plates and the tendency in the fluid to resist flow. When the force on the top plate is divided by the area of the plate, the shear stress applied to the fluid, σ, is found. For this geometry, the shear rate, $\dot{\gamma}$, is constant in the fluid, and can be calculated by V_0/h. So long as no-slip conditions hold on both plates, the shear rate depends only on the gap between the plates, the properties of the fluid, and the shear stress applied. For gaps much larger than many fluid molecules, the shear rate is independent of the gap. The ratio of the shear stress to the shear rate is the steady-shear viscosity, η. This property, with units of Newton-seconds per meter squared (Pa s), is readily calculated for a known geometry, force, and velocity.[189]

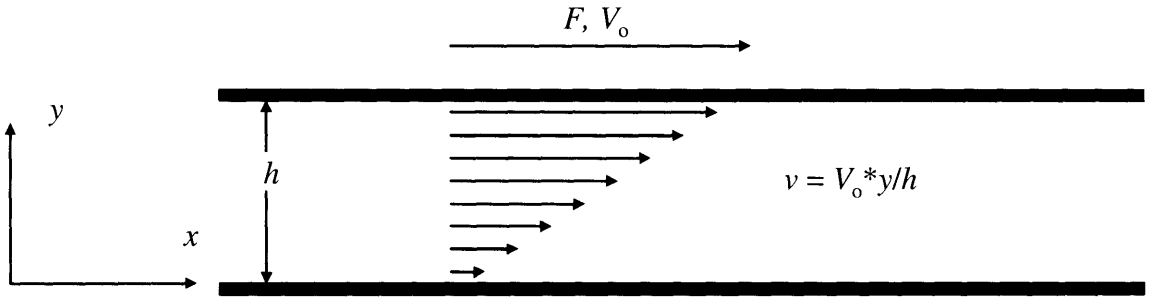

Figure 2.3.1 Schematic of steady shear viscosity in Couette flow With the bottom plate held fixed, the top plate reaches a steady-state velocity proportional to the applied force and the viscosity of the intervening fluid.

Steady shear viscosity is the basic rheological parameter, and is typically measured on an apparatus that approximates Couette flow. For simple fluids, η maintains a constant value over a wide range of conditions, and can be used as a means to characterize its resistance to flow. For more complex fluids, such as synovial fluid, η varies with shear rate. In the case of synovial fluid, η decreases as $\dot{\gamma}$ increases. At low shear rates, random molecular motion dominates, so large molecules, such as HA, become mechanically interlocked, or "entangled." Also, structures may form that favor intermolecular associations, such as protein-HA complexes. Both these interactions increase the fluid's resistance to flow. At high shear rates, large molecules become disentangled, and align preferentially in the direction of flow, minimizing resistance. When the flow is faster than the time scale of random motion, organized structures that resist relative motion cannot form, and resistance to flow decreases further. Consequently, η must be measured over a range of shear rates in order to represent the rheological properties of the fluid.[189] These measurements can be made on a device similar in principle to Fig. 2.3.1.

In order to characterize the fluid, it is appropriate to fit these results to a model, such that a few parameters can be compared among fluids. A model of shear-thinning that has been applied to synovial fluid[85] is the Cross model,[189] which fits η to the equation

$$\eta = \eta_0 / (1 + (c \cdot \dot{\gamma})^d) . \qquad \textit{Equation 2.3.1}$$

In this model, η_0 is the viscosity at low shear rate; c is the consistency, a measure of the relaxation time of the fluid; and d is the rate index, a measure of the shear-rate dependence of viscosity in the shear-thinning region.

Steady-shear viscosity and related parameters have been reported on a number of occasions in several patient populations. In the most comprehensive studies to date, Rainer *et al.* examined more than 200 *post mortem* and diseased samples,[85,94,190-193] though they did not report the size of the individual groups. Their results, as shown below in Table 2.3.1 with the results of a number of other groups, are considered the typical ranges for *post mortem* and diseased synovial fluid, and are generally consistent with the findings of others.[194] Each group includes a range of about one full order of magnitude. Notably absent from their reports is a comprehensive report of the viscous properties of joint fluid in the context of TJA.

Table 2.3.1 Viscous properties of synovial fluid Patient groups are described as they were in the original work. Joint fluid samples taken from knees. Degenerative and OA joints are considered equivalent, as are RA and inflammatory arthritis. Methods used are discussed in the text. Viscosity is presented in units of (Pa s), and consistency in (s). Rate index has no units. NR = not reported. *These values have been estimated from published charts, since the authors did not directly report the results. **Although the number of samples in each group is not reported, there authors reported a total of more than 200 samples examined.

Patients	*# of Samples*	η_0	c	d	*Reference*
Normal	2	$> 20^*$	$> 10^*$	0.75^*	Cooke, 1978[195]
Normal	NR	10 – 34	NR	NR	Safari, 1990[194]
Post mortem	NR**	6 – 12	40 – 100	0.7^*	Rainer and Schurz, 1980-1987[85,94,190-193]
OA	4^*	$0.1 - 1^*$	1^*	0.6^*	Cooke, 1978[195]
Degenerative	NR**	0.1 – 1	8 – 20	$0.3 - 0.6^*$	Rainer and Schurz 1980-1987[85,94,190-193]
RA	2	0.1	NR	0	Dintenfass, 1966[196]
RA	4^*	$0.02 - 0.1^*$	$0.1 - 1^*$	0.4^*	Cooke, 1978[195]
Inflammatory Arthritis	NR**	0.004 – 0.07	0.02 – 1	$0 - 0.3^*$	Rainer and Schurz, 1980-1987[85,94,190-193]
RA	NR	0.01 – 0.1	NR	NR	Safari, 1990[194]
TKA	2	$> 0.03^*$	$> 1^*$	$0.5 - 0.8^*$	Cooke, 1978[195]

The flow properties of synovial fluid are likely determined by HA molecular weight and concentration. As discussed in section 2.2.3, HA content varies in synovial fluid in both health and disease. Thus, HA content may explain much of the variability in flow properties reported, but this has not yet been shown directly. Chapter 4 of this thesis demonstrates the contribution of variability in HA concentration to the wide range of joint fluid viscosity. A relationship is supported by prior data in that healthy synovial fluid has greater viscosity, more HA, and higher molecular weight HA than OA or RA synovial fluid does. If protein adds to viscosity, this effect would lessen the differences among groups.

Since not all authors have used the same rheological model, it is difficult to compare different reports. Because Rainer, Ribitsch, and Schurz have conducted the most complete work and have used an appropriate model (the Cross model), other reports have been converted to this model when possible for Table 2.3.1. Since different groups have used different ranges of shear rate, certain Cross model parameters cannot be calculated. In such cases, lower bounds on these parameters are given. A limitation of the work or Rainer *et al.* is that they examined only *post mortem* samples from healthy joints. Since synovial fluid may become diluted after death,[95] their results likely underestimate the viscosity of synovial fluid from truly healthy joints.

Methodology Evaluation – Steady-Shear Viscosity

In 1966, Dintenfass summarized and reviewed the work to date by himself, Barnett, and Ogston on the viscous properties of human synovial fluid samples. He reported that RA fluids were primarily Newtonian, and fluids from other disease states exhibited shear-thinning, with higher viscosity.[196] He had used cone-on-cone geometry, and a very small amount (0.3 ml) of synovial fluid. He did not report what gap his apparatus employed.

In the same year, Davies *et al.* demonstrated shear-thinning in bovine synovial fluid from various joints. He found widely disparate values for the viscosity in different bovine joints.[197] These experiments were conducted using a cone and plate rheometer. In 1968 Palfrey and White reported similar results using an oscillatory apparatus, but found oscillatory experiments more challenging to interpret than steady-shear experiments.[198]

In 1968, Ferguson et al. compared synovial fluid from left and right knees of diseased (mostly RA) patients, finding an inverse correlation between the patient's perception of a "stiff joint" and synovial fluid viscosity.[172] In an interesting aspect of this study, the authors showed that viscosity of synovial fluid did not appear to change for the same patient throughout the day. This is the first and only longitudinal study on synovial fluid I have encountered.

In 1976, Reimann measured the viscosity of 80 pathological human synovial fluid samples at three different shear rates, finding the highest viscosity in joints with torn meniscus, and the lowest in RA and monarthritis. All samples showed some measure of shear-thinning.[7]

In 1978, Cooke evaluated the steady shear viscosity of a few normal and diseased human fluids over shear rates ranging from 0.1 s^{-1} to 1000 s^{-1}. He evaluated one normal post-mortem sample, and another two samples pooled together. These he found more than ten times as viscous as OA samples, which, in turn, were more viscous than RA samples. All of his experiments were conducted at 21°C, because he believed that synovial fluid would evaporate at higher temperatures.[195] Most interesting in this paper is the report of two TKA samples. The samples had quite different properties than all other samples evaluated, exhibiting a plateau region at high shear rate with $\eta_\infty \sim 0.02$ Pa s, and no evidence of a plateau at low shear rate to 1 s^{-1}. This result suggested that the inclusion of a high shear rate viscosity in the Cross model would be appropriate for joint fluid in TJA. The clinical outcome of these cases and the duration of implantation were not reported.

In 1980, Ly *et al.* examined synovial fluid from 59 patients with various arthritic conditions.[199] They used cone and plate geometry at 37°C, and did not report the shear range over which they measured. The highest shear rate they employed appears to be 150 s^{-1}. Each sample was fit to a power law model,

$$\sigma = K\dot{\gamma}^n . \qquad \textit{Equation 2.3.2}$$

Equation 2.3.3 can be manipulated to an equivalent shear-thinning portion of the Cross model, with $K = \eta_0 / c^d$ and $n = 1 - d$. This manipulation gives

$$\eta = \eta_0 / (c \cdot \dot{\gamma})^d , \qquad \textit{Equation 2.3.3}$$

which is equivalent to Equation 2.3.1 when shear rate is much greater than c^{-1}.

Ly separated his samples into "mechanical" and "inflamed" fluids using cytological measures, and the parameters K and n from the power law model. He found that for "mechanical" fluids, which he claimed were not different from normal synovial fluid, $K > 0.03$ and $n < 0.75$. These came from patients who had arthritic disease not related to changes in synovial fluid. On the other end, "inflammatory" fluids were much less viscous, and exhibited less shear-thinning ($K < 0.01$, $n > 0.85$). Ly did not report any statistical measure of the difference between the two groups, but he did report that there were five cases in which cytology and rheology were in discord. Furthermore, there were a class of fluids which fit the intermediate range, between "inflamed" and "mechanical." Fluids with parameters in between the two extremes were called mixed fluids. Ly reported an inverse relationship between K and n.

In the 1980s, Rainer, Ribitsch, and Schurz published a number of studies on the viscosity of normal synovial fluid.[94,190-192] These were summarized in English by Schurz in 1987,[85] and later in 1996.[193] They used two different rheometers, and so were able to cover the range of shear rates from 0.001 to 1000 s^{-1}. They did not report the gap in their experimental apparatus.

A number of experimental devices can be and have been used to determine the steady-shear properties of synovial fluid. These devices are typically limited, at low shear, by the resolution of the input motor or measurement device. Consequently, many of the results discussed above did not extend to the low shear rate plateau; η_0 was estimated from the data given. Devices have since become available that can measure rheological properties over up to three orders of magnitude of shear rates, though multiple devices are necessary to describe flow properties over a wider range. These devices should give similar results if properly calibrated, so the differences between each are not discussed in detail. The rheometer used in Chapter 3 to determine the flow properties of joint fluid in the context of TKA employs a double cylinder geometry that can be approximated as two parallel flat plates, as in Fig. 2.3.1.

2.3.2 Linear Viscoelasticity

In addition to the non-Newtonian behavior described above, synovial fluid has been shown to exhibit elastic properties, (*i.e.*, energy storage). It is computationally cumbersome to analyze the behavior of viscoelastic materials under the large deformations that may be relevant *in vivo*. For sufficiently small amplitude motions, however, the behavior of a viscoelastic material is independent of input amplitude, and can be characterized by a few parameters.[200] It is this linear viscoelastic range in which

researchers begin to characterize complex fluids, though relevant motion may occur at larger deformations.

A source of viscoelastic behavior in liquids is physical entanglement among long molecules. When subjected to small amplitude deformation, especially over short time periods, the network does not become disentangled. Rather, the chains are stretched and compressed, thus storing energy like a solid under elastic deformation. Larger motions and longer time periods do invoke molecular disentanglement, however. Thus, elastic behavior in liquids is most prominent in small amplitude, high velocity motion. In a human joint, this situation corresponds to actions such as running or jumping (~ 3 Hz). Walking occurs at closer to 1 Hz, and other actions, such as standing, can have much longer characteristic times. It is believed by some that the viscoelastic properties of synovial fluid serve to support and protect joint tissue under high velocity motion.[126] Joint fluid may serve a similar function in TJA.

Viscoelastic behavior can be modeled most simply as a spring and damper in series (Maxwell model) or in parallel (Kelvin model). Neither of these represents the behavior of real materials as completely as more complex models of springs and dampers in series and parallel. Even the most complex networks of springs and dampers can be approximated by a single viscous parameter and a single elastic parameter for a given frequency, however. Thus, viscoelastic behavior is described by a single frequency-dependent elastic modulus, G', and a single frequency-dependent viscous modulus, G''.

Viscous and elastic parameters are measured under oscillatory motion using the same Couette geometry as is used for steady-shear viscosity (Figure 2.3.2). An oscillatory strain

$$F = F_0 \cos \omega t, \qquad \textit{Equation 2.3.4}$$

is applied to the top plate. Since either model leads to the same constitutive equation, only the Kelvin model is calculated here. The applied force is equal to the sum of the force applied to the spring and that applied to the damper (*i.e.*, $F = F_{spring} + F_{damper}$). Since the areas over which these forces act are the same, the shear stresses (σ) obey the same relationship. Using the Newtonian and Hookean relations for the spring and damper, respectively, we find

$$\sigma = \sigma_0 \cos(\omega t) = G'\gamma + \eta'\dot{\gamma}, \qquad \textit{Equation 2.3.5}$$

where σ_0 is the shear stress amplitude, G' is the storage modulus, η' is the dynamic viscosity, γ is the strain, and $\dot{\gamma}$ is the strain rate. The viscous parameter can also be described by $G'' = \eta'\omega$.[200]

For sufficiently small amplitude motion, shear output is sinusoidal with the same periodicity as the input, though out of phase. The shear strain (elastic component) and shear rate (viscous component) can be decomposed using $\gamma = \gamma_0 \cos(\omega t - \phi)$ and its derivative, $\dot{\gamma} = -\gamma_0 \omega \sin(\omega t - \phi)$, where γ_0 is the shear strain amplitude and ϕ is the phase delay of the deformation output. Combining these equations with Equation 2.3.5, and using an identity relationship for the cosine, we find

$$\sigma_0 \left(\cos(\omega t - \phi)\cos\phi - \sin(\omega t - \phi)\sin\phi\right) = \gamma_0 \left(G' \cos(\omega t - \phi) - G'' \sin(\omega t - \phi)\right). \qquad \textit{Equation 2.3.6}$$

Decomposing this equation into sine and cosine, $G' = \sigma_0/\gamma_0 \cos\phi$ and $G'' = \sigma_0/\gamma_0 \sin\phi$.

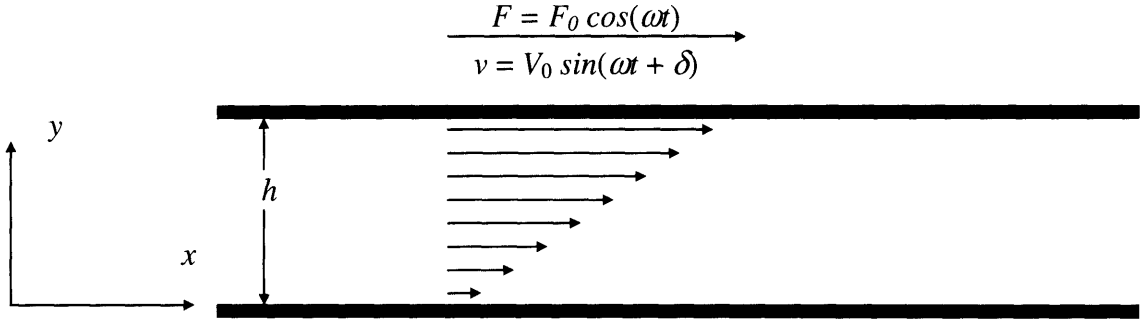

Figure 2.3.2 Schematic of small amplitude oscillatory shear Under small-amplitude oscillatory shear stress, the top plate moves in a sinusoidal pattern out of phase with the input. The phase and amplitude of response are related to the viscous and elastic character of the fluid.

Thus, these parameters describe the relative importance of elasticity and viscosity in small amplitude oscillatory motion. In many viscoelastic fluids, low frequency motion is dominated by viscous behavior (*i.e.*, $G' < G''$), whereas, at high frequency, elastic behavior dominates (*i.e.*, $G' > G''$). For these fluids, the frequency at which storage and loss moduli are equivalent is called the crossover frequency, f_c. This parameter is useful in characterizing fluid behavior because the fluid acts more solid-like at frequencies higher than f_c and more fluid-like at frequencies less than f_c.

A number of researchers had demonstrated the elastic nature of animal synovial fluid as early as 1950.[112]. Anecdotal reports date to 1966, but Anadere, in 1979, was one of the first to quantify this property in human synovial fluid in healthy and diseased joints. He evaluated linear viscoelasticity only at 2 Hz, and found that normal synovial fluid had higher viscous and elastic properties than OA and RA samples.[77] Earlier reports were generally consistent with this finding.[201] Notably, normal and OA fluid had greater elastic modulus than viscous modulus at 2 Hz, but RA samples did not.

By far, the most comprehensive study in this area has been conducted by Balazs,[80] who believed that the viscoelastic nature of synovial fluid served to protect articular cartilage from damage. Balazs examined many patients at a variety of ages, and found that the viscoelastic properties of healthy synovial fluid decline as patients age, even in the absence of symptomatic joint disease.[80] He reported f_c as well as G' and G'' at 2.5 Hz. He found that f_c increases with age (from 0.1 Hz in young adulthood to 0.4 Hz after age 50) while the moduli at crossover decreases (from 30 Pa in young adulthood to 6 Pa after age 50). While these properties were all "degenerate" in older patients, they were further altered in OA patients, in whom crossover occurred at 4 Hz.[80] The results of Balazs differ markedly from the report of Anadere. A summary of previous work on viscoelasticity in synovial fluid is given below in Table 2.3.2.

Safari *et al.* reported viscoelastic moduli from synovial fluid of healthy and diseased patients over a range of frequencies, though they only tabulated their results at 2 Hz, and did not report their sample sizes.[194] They showed results similar to Anadere with regard to crossover frequency, with normal samples having crossover at 0.2 to 1.5 Hz, and RA samples with crossover frequency 30 Hz or higher.

Table 2.3.2 Viscoelastic properties of synovial fluid Patient groups are described as they were in the original work. Joint fluid samples taken from knees. Degenerative and arthritic joints are considered equivalent. Methods used are discussed below. Storage and loss moduli are presented in units of (Pa). Data are presented as mean ± standard deviation or range, as presented in the original work. When necessary, standard error of the mean has been converted to standard deviation. y.o. = years old; NR = not reported. *Results were given in "dynes/sec^{-2}," which is assumed to be an editing error for dynes/cm^2 (0.1 Pa), based upon the accompanying graph.

Patients	*# of Samples*	*Freq.*	*G'*	*G"*	*Reference*
18 –27 y.o.	16		120 ± 10	45 ± 8	
27 – 35 y.o.	18	2.5 Hz	22 ± 1	7.2 ± 0.8	Balazs, 1982[*80]
52 – 78 y.o.	26		19 ± 3	10 ± 1	
Normal	NR	2 Hz	1.6 – 2.8	0.7 – 1.0	Safari, 1990[194]
Meniscus Defect	7	2 Hz	0.10 ± 0.03	0.08 ± 0.03	Anadere, 1979[77]
Meniscus Defect	NR	2 Hz	0.01 – 0.2	0.03 – 0.2	Safari, 1990[194]
Degenerative	12	2 Hz	0.72 ± 0.06	0.62 ± 0.03	Anadere, 1979[77]
OA	11	NR	8.5 ± 5.4*	4.8 ± 2.8*	Balazs, 1982[80]
RA	34	2 Hz	0.02 ± 0.01	0.03 ± 0.02	Anadere, 1979[77]
RA	NR	2 Hz	0 – 0.05	0.01 – 0.08	Safari, 1990[194]

Other groups later reported these moduli at several frequencies from pathological fluids.[82,202] These results were similar to those obtained previously, but are not amenable to tabulation in the format of Table 2.3.2. Some groups found crossover for OA fluids, as Balazs had, but at least one group found no crossover in a large proportion of OA samples.[124]

It is believed by many that one of the functions of synovial fluid is to cushion the synovial joint from the impact of high energy motion, such as in running or jumping.[126] Within this framework, the elastic properties of synovial fluid would enhance the maintenance of separation between the cartilage surfaces.[18] Lai *et al.* compiled a thorough list of equations that could be used to describe the flow of synovial fluid, including a relaxation spectrum.[203] It is not clear that these equations will facilitate a quantitative flow analysis in human joints such as the knee and hip because of the complexity of motion. Qualitatively, however, the elastic properties of synovial fluid are consistent with this view: synovial fluid from healthy joints exhibits more elasticity than synovial fluid from diseased joints, though these degenerated properties have not been shown to be the cause of joint disease. This protection may carry over to TJA, in which joint fluid with greater elastic properties may offer more protection for articulating prosthesis surfaces than joint fluid with degenerated elastic properties.

Notably absent from this table is a description of the viscoelastic properties of joint fluid in the context of TJA. To our knowledge, there are no previous reports of these data. These data are necessary to evaluate whether viscoelastic properties may play a meaningful role in the lubrication of TJA. The compilation of these data for comparison with those of Balazs is among the main purposes of Chapter 3 of this thesis.

Methodology Evaluation – Linear Viscoelastic Properties

In fashion similar to experimentation on the steady shear viscosity of synovial fluid, a number of devices have been used to determine the viscoelastic properties of

synovial fluid. These apparatus are available commercially, and tend to use the parallel plate model described in Fig. 2.3.3, though other arrangements have been used. Data gathered on these devices should be independent of the experimental apparatus, so long as appropriate geometry is used. For convenience, the same device can be used to determine steady shear viscosity and linear viscoelastic parameters.

2.3.3 Thixotropy and Anti-thixotropy

In addition to the above non-Newtonian effects, there is evidence that the viscosity of synovial fluid can change with time under steady shear. When viscosity decreases with time under shear, the fluid is said to be thixotropic;[189] when it increases, anti-thixotropic or rheopectic (though some researchers refer to shear thinning and thixotropy interchangeably[6,23]). Such behavior might be relevant in the tribology of replacement joints: if the properties of the fluid were such that resistance to flow increased substantially in the absence of motion, a squeeze film effect could enable a sufficient fluid film even under static or near-static circumstances, when fluid film lubrication usually would not occur.

In 1966, Davies first suggested that synovial fluid might have thixotropic properties based upon the observation that the fluid exhibited a high initial resistance to shear flow after a period of rest. The magnitude of the initial resistance appeared to depend on the duration of rest prior to shearing.[197] Such behavior would suggest an interaction between components of synovial fluid that may support fluid film lubrication even during stasis. Increased resistance to flow under small shear would augment a squeeze film during standing.

The report of Oates *et al.* supports this finding. They found thixotropic behavior in a model joint fluid composed of HA (molecular weight 1.6 MDa), albumin, and γ-globulin. Both the protein solutions and the synthetic synovial fluid exhibited increasing viscosity with time at low shear rates (0.05 s^{-1}) after shear at high rates.[119] While this behavior could be considered rheopectic, since it refers to increasing viscosity with time under shear, the model of HA-protein interaction interrupted by high shear is consistent with the above explanation of thixotropic behavior. This finding could thus be considered thixotropic in that high shear rate reduces the apparent viscosity, which then recovers with time under reduced shear rates.

Other groups have shown thixotropic behavior of unknown significance in synovial fluid; their work is included for completeness. In 1990, Safari *et al.* showed slow recovery of viscoelastic moduli in small amplitude oscillatory shear after steady shear at high shear rates.[194] In 1996, O'Neill *et al.* reported a 10% increase in viscosity over the course of five minutes for OA synovial fluid under steady shear at temperatures below 25°C. Above this temperature, this behavior was not observed. This behavior was confirmed by hysteresis loops at 0°C and 5°C using a continuous shear stress ramp from 0 to 2 Pa (0 to 25 s^{-1}). Finally, by vigorous shaking of the sample, a foam was created with viscosity four times that of the fluid prior to shearing.[204] The authors do not report a shear history of the synovial fluid samples prior to their experiments. It is not clear that behavior observed at close to freezing but not at physiological temperatures has much relevance *in vivo*. Likewise, the description of foam formation seems unlikely to occur

clinically, though the authors suggested that these foams may form clinically in the case of high-impact activity.

Finally, In 1985, Altmann *et al.* showed hysteresis loops in inflammatory synovial fluid, but not normal fluid, due to the presence of fibrinogen.[205] As discussed in section 2.2.1, fibrinogen is not normally present in the joint space, but could be in certain inflammatory states due to synovial membrane dysfunction. Not surprisingly, the presence of fibrinogen can lead to gel formation upon extended stasis (more than 6 hours). It is not clear how these gels would apply to the tribology of natural or replacement joints, though one could imagine either a positive or negative contribution in both articulations.

In the examination of the flow properties of joint fluid in the context of TKA (Chapter 3), this thesis examines the findings of some of these authors regarding the thixotropic properties of joint fluid.

2.3.4 Normal Stress Differences

Another rheological parameter that may relate to fluid film lubrication is the first normal stress difference. Found in many viscoelastic fluids, normal stress differences arise from anisotropy induced by steady shear flow. A normal stress difference is a difference between a stress exerted by the fluid in a direction perpendicular to flow and the stress exerted in the direction of flow. The first normal stress difference, N_1, can be visualized from Fig. 2.3.1, as the difference between the stress along the y-axis and that along the x-axis. At low shear rates, this stress difference tends to be proportional to the square of shear rate. As shear rate increases, this stress difference tends to reach an upper limit, and then declines at higher shear rates. Thus, the shear stress required to produce some intermediate shear rates can be greatly exceeded by N_1 in certain elastic fluids.[189] If this property dominates in synovial fluid at physiological shear rates, normal stress differences could contribute to load bearing in the synovial or replacement joint, thus maintaining a sufficient gap for fluid film lubrication.

Davies measured normal stress differences in synovial fluid from bovine joints in 1966.[197] He found peak stress differences up to 80 Pa at startup, and up to 25 Pa under steady shear. These stress differences were still increasing at the highest shear rates measured, 1400 s^{-1}. In 1968, Ferguson e*t al.* reported comparable findings from normal human knees *post mortem* and OA knees, up to 200 Pa under steady shear of 450 s^{-1}.[172]

In 1987, Schurz reported N_1 in a limited number of samples of human synovial fluid.[85] The highest values he obtained in healthy fluids were on the order of 500 Pa at shear rates of 500 s^{-1}, though, again, the values were still increasing at the highest shear rates measured. Although this value is an order of magnitude higher than the shear stress required to generate steady flow at a shear rate of 500 s^{-1}, this stress cannot nearly support the stresses encountered in both natural and replacement[206] knees and hips. Normal stresses would have to increase greatly as the gap between surfaces decreases to that found in the replacement to be relevant in the tribology of replacement joints.

2.3.5 Extensional Viscosity

Another potentially relevant property exhibited by synovial fluid is extensional viscosity. Consider two parallel plates, as in the Couette example of Fig 2.3.1. Now,

instead of applying a transverse force, consider a force along the y-axis pulling the plates apart. Extensional viscosity relates to the fluid's resistance to this type of motion.[189] Extensional flow likely occurs in many situations in replacement joints. Examples include the fluid motion generated by a femoral condyle rolling on the tibial plateau and the fluid motion in squeeze film lubrication.

In 1996, al-Assaf *et al.* showed that extensional viscosity dominated over shear viscosity in HA at shear rates above 500 s^{-1}.[188] No corresponding study has been published on the extensional rheology of joint fluid, to my knowledge.

2.3.6 Piezoviscous Effect

All the properties discussed above have been considered under conditions of atmospheric pressure. The synovial joint maintains a sub-atmospheric pressure (as low as 650 mm Hg) that may help to maintain joint stability even in the absence of load. This reduced pressure may be vital to proper function of the synovial membrane, so the pressure difference, which can be lost in inflammatory states,[72] may affect the quantity and composition of joint fluid. Thus, this type of pressure change can indirectly affect the tribology of the joint. Changes in hydrostatic pressure on this scale are unlikely to directly affect the flow properties of joint fluid.

Under high loading conditions, however, local pressure variation may have a meaningful effect. For many fluids, viscosity has been found to increase exponentially with pressure according to

$$\eta_p = \eta_0 e^{\alpha p}, \qquad \textit{Equation 2.3.7}^{13,189}$$

where η_0 is the viscosity at atmospheric pressure, p is the pressure, η_p is the viscosity* at pressure p, and α is the pressure-viscosity coefficient. For most fluids, α is a very small number: for mineral oil, α is on the order of 10^{-8} Pa^{-1}. Therefore, it takes pressures in the hundreds of megapascals to affect η for these lubricants. For this reason, piezoviscous effects have been assumed by many to be negligible within the range of physiological pressures[21,31] (up to tens of megapascals).[206]

Webb and Stachowiak actually evaluated the pressure-viscosity coefficient of five synovial fluid samples from OA and RA patients at very high shear rates (~10^6 s^{-1}) at 5 and 15°C.[207] They found, at 15°C, that α is on the order of 10^{-5} Pa^{-1}; thus, at low temperatures, the viscosity of synovial fluid increases 22,000-fold at a pressure of 1 MPa. As pressures increase more, viscosity would continue to double with every 70 kPa increase in pressure. If this relationship holds at physiological temperature, stress, and gap, this piezoviscous effect would substantially affect squeeze film and EHD lubrication in both natural and replacement joints.

Webb and Stachowiak conducted these experiments over a small range of shear rates, pressures, and temperatures. Although the pressure-viscosity coefficient decreased by 5-10% in three out of five cases when the temperature was increased from 5°C to 15°C, the higher temperature values are still an order of magnitude greater than those typically found in industrial lubricants.[13] Due to the exponential nature of the pressure-

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* This viscosity is not to be confused with η_{1Pa}, the viscosity at 1 Pa shear stress. Both expressions have subscripts with units of Pascals.

viscosity relationship, an order of magnitude increase in this parameter corresponds to a much larger increase in piezoviscous effect. The authors did not show conclusively in this work that the pressure-viscosity relationship obeyed Equation 2.3.7 up to physiological pressures, however, and did not report the pressure range in which they measured. Although a worthy enterprise, it is outside the scope of this thesis to measure this property in joint fluid in the context of TJA.

2.3.7 Temperature Effects

Although it has not been much noted in the above discussion, temperature affects the viscosity of synovial fluid. It is noted empirically (with some molecular basis) that the rheological behavior of many polymer solutions depends on temperature as it depends on time.[189] Thus, rheological behavior can often be graphed as a modulus versus a time-temperature coefficient on a master curve, covering a wide range of temperatures and shear rates (so long as the polymer does not change phase or conformation). This relationship has been shown, and such curves generated, in HA solutions.[105]

The relationship between temperature and viscosity in joint fluid was noted over the range from 25°C to 40°C in my master's thesis;[136] viscosity decreases as temperature increases (it roughly reduces by half in that range). Consistent with this result, Webb and Stachowiak reported, in the same study that examined the piezoviscous effect, a decrease in viscosity by 2.5 to 5 times over the range 5°C to 35°C.[207]

Most researchers in the field have evaluated the rheology of synovial fluid at close to 25°C. Some researchers have claimed that synovial fluid evaporates quickly at physiological temperature, but my experience was that protein denaturation and precipitation was a greater problem than evaporation. Furthermore, elastic properties were difficult to measure at higher temperatures, presumably owing to the time-temperature superposition effects. Thus, measuring these properties at 25°C is deemed most appropriate to conveniently compare among samples and with prior work by others.

2.3.8 Small Gap Rheology

The discussion above is based on a continuum understanding of joint fluid; that is, the fluid is treated as a continuous medium, rather than a collection of particles. This assumption holds until one reaches the molecular scale, wherein a discrete model may be more appropriate. A notable consequence of the continuum approximation is that rheological properties are independent of the gap between surfaces. This assumption is necessary to use the most convenient tools for rheological analysis, but results must be applied carefully when smaller gaps are possible.

In the natural joint, the gap falls to 0.1 μm or less in places,[10] which is on the order of the length of a 500 kDa HA molecule.[26] Clearly, the continuum model cannot be applied everywhere in the natural joint. In the replacement joint, since surfaces come into contact, the gap must drop to very nearly zero in places. Even when portions of the surface are separated by a single molecule, gaps in other portions of the articulation dictate a continuum approximation. Therefore, the information gained from these large (hundreds of microns) gaps has significance for the replacement joint. Nonetheless, the gap must be considered in all rheological analyses of synovial fluid. In many studies, especially older ones, the gap between surfaces was not reported.

Tadmor *et al.* recently examined the rheology of physiological HA solutions under small gaps.[26] They found that the solution maintained its bulk viscosity until the film decreased to 400 nm, wherein the viscosity dropped monotonically to that of the solute. The authors reported that the "extruding" out of HA could contribute to fluid film lubrication, reinforcing the importance of squeeze films. The authors also found that HA was a poor boundary lubricant, as suggested by many others in cartilage-on-cartilage and latex-on-glass friction experiments. In order to achieve very small gaps, however, the authors had to use mica surfaces, which likely interact with HA in a manner different than cartilage or replacement joint materials. It would be interesting to compare the behavior of components of joint fluid on more relevant surfaces, if small gaps could be achieved between them.

2.3.9 Summary of Joint Fluid Flow Properties

In summary, there are many flow properties of joint fluid that *could* be relevant in the lubrication of replacement joints. Since it is not possible to fully characterize the behavior of this complex fluid under all flow conditions, it is reasonable to begin with the basic and commonly-measured flow properties, steady-shear viscosity and linear viscoelasticity. These properties cover the two major behavioral regimes of joint fluid (liquid-like and solid-like), and enable for maximal comparison with previous work and with theoretical understanding. Furthermore, although small gap rheology likely plays an important role, the same reasons given above make bulk rheology an appropriate target of research.

Neither viscosity nor linear viscoelasticity have been characterized in the context of TJA, though both have been demonstrated and, to an extent, quantified, in the natural joint. There is reason to speculate that these properties are different in the context of TJA. These properties are degenerate in joints of patients with certain arthritic conditions, conditions that often eventually lead to joint replacement. Second, the synovial membrane, which produces and/or filters the components of synovial fluid, is removed during joint replacement surgery. The repaired/regenerated membrane may function quite differently.

Finally, while it is believed that rheological properties are relevant in the natural joint, it is not clear whether they affect current replacement joint technology. Future TJA technology may depend more highly on fluid film mechanisms for tribology, however, and may even be driven by an improved understanding of the rheology of joint fluid. Chapter 3 of this thesis quantifies the viscous and linear viscoelastic properties of joint fluid in the context of TJA.

2.4 Engineering Tribology and TJA

There have been many examinations of the wear of PE in TJA. These studies have begun to bridge traditional engineering tribology and this new articulation and environment, but have largely been conducted without consideration of the topics discussed above, including lubrication by joint fluid. These studies include analyses of clinical data, but rely heavily on the generation of laboratory data through wear and friction experiments. This section begins with a brief summary of traditional approaches to tribology as they have been applied to TJA. This section continues with a discussion

about methods of clinical evaluation of TJA and three types of laboratory wear experiments adapted to simulate replacement joint articulations. This section closes with an overview of the development of tribological studies specifically concerned with the role of lubricant.

2.4.1 Traditional Approaches to Tribology in TJA

Many different mechanisms have been proposed to explain the generation of wear. Reducing wear in a given articulation begins with identifying the dominant wear mechanism in that articulation. Abrasive, adhesive, and a type of delamination wear all appear to be relevant to the tribology of TJA. For each of these mechanisms of wear, models have been proposed which relate volumetric wear (V) to parameters of the articulation. In each case,

$$V = l \times W \times k, \qquad \textit{Equation 2.4.1}$$

where l is the total distance slid, W is normal load, and k is a constant determined by the materials and surfaces in contact.

Abrasive Wear

One mechanism of wear involves the plowing of a surface either by a harder counterface or by particles trapped between the surfaces.[13,208] This abrasive form of wear has been analyzed in a number of metal-on-metal articulations: equations have been developed (with some empirical support)[209] relating wear to sliding distance, hardness of the softer surface, and normal load. For example, one abrasive wear model employs a force balance on a single asperity, then sums over all asperities. In the form of Equation 2.4.1, this model predicts

$$V = l \times W \times C \times \tan\theta / \pi H, \qquad \textit{Equation 2.4.2}$$

where C is a constant related to the "efficiency" of wear, H is the hardness of the softer surface, and θ is the angle of an asperity on the harder surface.[13]

Other models have been developed for abrasive wear, taking forms similar to that of Equation 2.4.2, with volume of wear generated proportional to sliding distance and load and inversely proportional to the hardness of the softer surface.[208] Evidence of abrasive wear *in vivo* includes burnished appearance of the PE surface on gross examination and scratches microscopically.[210] These have been found in both THA and TKA. Furthermore, abrasion of the femoral head by third bodies embedded in acetabular cups has been found in retrieved THA.[211]

Lubricants can serve several protective functions in abrasive wear. Obviously, if fluid film lubrication takes place, there is no surface-surface contact, and no chance for plowing. If fluid film lubrication is not possible, a lubricant can remove particles and prevent wear particle agglomeration,[208] thus reducing wear by third body abrasion. In TJA, third body wear is recognized as a potential source of PE wear particle generation. A number of studies have sought to evaluate the ability of various couples to resist third body wear.[212] Boundary lubrication theory suggests that adsorbed components can repel opposing surfaces, thus reducing plowing.[1,13] Energy generated in friction may be dissipated by shearing off boundary lubricant layers, thus reducing the total amount of energy dissipated by deforming the softer surface. It is not clear, however, that this effect is significant.

Adhesive Wear

In adhesive wear, particles from one surface bond to the other. When the surfaces are pulled apart, the bond between particles on opposing surfaces is stronger than the bond between particles on the same surface, and a particle is transferred to the other surface.[13] Adhesive wear theory predicts a similar relationship between volumetric wear, hardness, load, and sliding distance:

$$V = l \times W \times C/3H \, . \qquad \textit{Equation 2.4.3}^{208}$$

Although some scientists question the validity of the adhesive wear model,[208] most sources cite adhesive wear as an important component of PE wear in TKA and THA.[213]

As in abrasive wear, fluid film lubrication would prevent adhesive wear by preventing contact between the surfaces. When fluid film lubrication is not possible, a boundary layer of adsorbed components may protect against adhesive wear by blocking molecular scale contact, thus physically preventing adhesion. Alternatively, an adsorbed molecule could reduce the energetic favorability of bonding between surfaces.

Adhesive wear can result in the deposition of a transfer film, in which material from one surface is deposited on the other. Transfer films have been seen in laboratory wear tests of metal-on-PE, especially when distilled water is used as a lubricant,[214] but transfer films have not been seen clinically.

Pitting, Fatigue, or Delamination Wear

The third type of wear relevant in replacement joint articulations differs from adhesive and abrasive wear in that it involves damage that penetrates the articulating surfaces. Suh called this type of damage "delamination" wear, hypothesizing that high surface loads generate subsurface cracks that propagate and eventually reach the surface, generating wear sheets.[215] Although this description is not consistent with wear particles encountered in laboratory tests simulating replacement joint articulations, it is likely that processes similar to those described by Suh sometimes cause PE damage in TKA. Bartel and Wright described this type of wear as a fatigue process, and preferred to call it "surface damage" rather than wear.[216] Collier *et al.* have shown this damage to be associated with thin PE components.[217]

It is theorized that such damage occurs only when local stresses exceed the yield stress of the material, leading to plastic deformation. The maximum stresses in metal-on-PE replacement hips and knees have been estimated using finite element modeling[216,218] and pressure-sensitive film.[219] In the hip, maximum compressive stresses have been reported at approximately 15 MPa. The same study reported maximum compressive stresses in the knee on the order of 40 MPa. Tensile stresses of 5 MPa and 15 MPa occurred in the hip and knee, respectively. Unbalanced loading increased these tensile stresses. Metal-on-metal prostheses generated still higher stresses, estimated at close to 40 MPa in THA by finite element analysis.[220] It has been reported by Collier *et al.* that stresses present in the replacement knee joint exceed the uniaxial yield stress of PE,[221-223] particularly in less conforming knee designs. Using pressure-sensitive film, they showed that even patellar components can, in flexion, experience compressive stresses greater than the yield strength of PE.[219,222] The compressive yield strength of PE is on the order of 20 MPa,[19] lower than the maximum compressive stresses found in TKA and on the order of the maximum stresses in THA, confirming the suggestion of Collier *et al.* These

results show that the kind of subsurface damage described separately by Suh and Bartel can occur in metal-on-PE prostheses, particularly in TKA.

Both finite element modeling and pressure sensitive film may underestimate the *actual* applied stresses at asperity contacts. Finite element modeling assumes smooth surfaces, and so may presume larger contact areas than actually exist. Pressure sensitive film, having some thickness, acts to smooth out the surfaces, and may also enlarge the area of contact. Atomic force microscopy has measured local stresses in PE on Co-Cr in the hundreds of megapascals.[224] Thus, although the actual applied stresses in replacement joints are not known, it is clear that these stresses can exceed the yield stress of PE.

This type of wear has also been analyzed quantitatively in metal-on-metal articulations, and equations like 2.4.2 and 2.4.3 have been generated.[208,225] This mode of wear results in wear sheets much larger than the particles generated by other wear mechanisms, but only occurs when local stresses greatly exceed the yield strength of the counterface. Finite element modeling of TJA suggests this criterion to be likely in TKA but not THA,[216] though the true stresses at asperity contacts in these articulations are not known. Nonetheless, wear morphology more characteristic of delamination than abrasion or adhesion has been seen in some cases of TKA.[226,227]

The stresses required to generate this type of wear likely occur only at asperity contacts, so their presence implies that fluid film lubrication is not taking place. Conversely, if fluid film lubrication did occur, it would distribute the load more evenly on the surfaces, reducing this subsurface damage. Boundary lubrication is unlikely to significantly affect delamination wear. Efforts at preventing subsurface damage typically involve modifying materials and geometry to reduce the stresses applied to the surfaces, without consideration for the effect of lubricant.

It is not appropriate to take the equations derived empirically in one type of articulating couple (metal-on-metal) and apply them to a different couple (metal-on-PE). First, a mechanism that dominates in a copper-on-steel articulation may be irrelevant to Co-Cr on PE, even under the same loading conditions. Second, the hardness of PE, unlike that of metals, changes over time under load, hampering the application of the equations including that parameter.[208] Nonetheless, Equation 2.4.1 has been universally employed to describe the wear of polymers,[228] with k being known as the wear factor, an empirically-determined constant. In the absence of better understanding of wear in TJA, adhesive, abrasive, and delamination wear are lumped together and considered empirically under the umbrella of Equation 2.4.1. Use of this equation in the clinical literature is discussed below.

Friction

Despite its important role as a marker of tribological performance, friction in TJA has received relatively little attention in the research community. In complex joint simulators, complex motion and dynamic loading make it difficult to measure a meaningful coefficient of friction. On the other hand, it is not easy to apply the friction generated in a simple unidirectional pin-on-flat articulation to the generation of wear particles in complex articulations. Nonetheless, friction is the starting point of tribology, and therefore is an essential part of a discussion of the tribology of TJA.

Assuming mixed or boundary lubrication, frictional force in this articulation is primarily generated by plowing of the softer surface by the harder.[229] This is certainly true of hard-on-hard articulations when two body abrasive wear dominates, and is true for metal-on-PE at physiological speeds even if adhesion contributes to wear. This force can also be called "the force required to shear junctions" between asperities,[230] and is sometimes expanded to include the force of shearing a boundary lubricating molecule.

Friction obeys general relationships with load, velocity, and contact area, though for specific surfaces under specific conditions, these relationships may not hold true. The general relationships, as applicable to TJA, are as follows:

(1) Friction is proportional to A_r, the real area of contact.[230] Under plastic deformation, as in metal-on-metal articulations, the additional relation $A_r = W/H$, where W is normal load and H is hardness, leads to the linear relationship between friction and normal load, and to the use of a "coefficient" of friction. Under elastic deformation, as in Hertzian contact, the real area of contact is proportional to load to the 2/3 power, so the "coefficient" of friction decreases with load. In metal-on-PE, the relationship between load and friction typically falls somewhere between these extremes.[231]

(2) Friction is independent of velocity.[230] Under boundary lubrication conditions, the rate of motion does not directly affect the energy dissipated per unit sliding distance, though under high velocity or load, surface heating[208] and viscoelastic effects[232] could affect the tribology of the interface. Such changes are not likely to be significant under physiological conditions.

Friction is important because it is a source of abrasive wear and an indicator of adhesive wear. Very high friction (*i.e.*, seizure) could lead to pitting and delamination, but these could also occur due to high normal loads in the absence of high friction. A more detailed discussion of friction in TJA is given in Chapter 5, in which friction is used to rank lubricants and couples for use in TJA.

2.4.2 Empirical Results in Vivo

The first hard on soft replacement joints, implanted by Charnley in the late 1950s,[233] consisted of stainless steel femoral heads articulating in poly tetrafluoroethylene (PTFE) acetabular cups. PTFE was chosen because of its excellent coefficient of friction in the absence of lubricant.[234] It had been observed that, when PTFE articulates on another surface, a small amount of PTFE binds to the counterface within a short time. Then, it is believed that natural repulsion of fluorine atoms lubricates the surface, providing a coefficient of friction of about 0.1.[208] Consequently, PTFE provides its own solid lubrication, and joint fluid lubrication was not considered. It was quickly discovered that PTFE generated clinically unacceptable wear rates, and Charnley replaced PTFE with the relatively wear-resistant PE, which performed much better.

Since then, clinicians and researchers have been continuously evaluating existing and alternative bearing surfaces in hopes of improving the performance of replacement joints. A number of clinicians and researchers have evaluated TJA designs by assessing their success and failure *post hoc* – by retrieving and analyzing prostheses either *post mortem* or after revision surgery. Many such studies have been conducted; only two are discussed here.

In 1985, Atkinson *et al.* used a casting technique to estimate the volumetric wear in 25 hip prostheses retrieved during revision surgery. They calculated a wear factor, k, using Equation 2.4.2. The authors estimated l from femoral head size and an empirical equation dependent on the patient's age; W was taken as the patient's weight. They found k to be $2.9 \pm 2.3 \times 10^{-6}$ mm^3/Nm, with values ranging from less than 0.09 to 7.2×10^{-6} mm^3/Nm.[235]

In 1996, Hall et al. conducted a similar work on a group of over one hundred hips undergoing revision surgery. They calculated k to be $2.1 \pm 2.3 \times 10^{-6}$ mm^3/Nm, confirming both the mean and range of the previous work.[236] Such wide spreads in data, reflected by both range and standard deviation, suggest that factors other than sliding distance and load determined the wear rate in these hips. These factors may include stresses, area of contact, surgical technique, patient gait, and lubricant quality and quantity. One limitation of the results of these studies and others like them is that these joints were retrieved from failed prostheses, and therefore the group may contain some ascertainment bias. Other studies of prostheses that did not require revision have shown little or no wear, even after many years *in vivo*.[214] These studies remind us that the true *mean* wear rate likely differs from the wear rate among joints that eventually require revision. Including date from successful prostheses would only extend the range of wear rates measured. These studies indicate the limitations in our understanding of what determines PE wear in these articulations.

A means to study wear in TJA nondestructively is to analyze successive radiographs *in vivo* over the course of years. In 1990, one group reported a mean volumetric wear of 0.1 mm^3/year in THA and found an association between volumetric wear and bone resorption.[237] Larger (32 mm) acetabular cups have been associated with higher volumetric wear rates than 28 mm and 22 mm cups in this and other such studies. Higher body weight was correlated with greater volumetric wear, but the correlation coefficient, R^2, was only 0.10 for body weight, so the relationship may be an artifact of a relationship between body weight and acetabular cup size. This result calls into question the validity of relating volumetric wear linearly with patient weight.

Schmalzreid *et al.* examined 11 hip prostheses removed at autopsy, finding evidence of loosening even in the absence of radiographic evidence.[238] This study also supported a correlation between volumetric wear and loosening.

Collier and coworkers examined the tibial inserts of 122 retrieved knee prostheses, finding gross wear on 75 of them (62%).[221] Using pressure-sensitive film, they demonstrated, in knee flexion, contact stresses in excess of the yield strength of PE. Their results showed that less congruous designs resulted in high stresses (~45 MPa) greatly exceeding the yield strength of PE; consequently, they recommended the use of more conforming designs. This recommendation is reflected in meniscal bearing TKA designs, which contain a third piece, a bearing between the tibial plateau and femoral condyles. There is some dispute as to whether contact stresses are actually reduced in these bearings,[239] but it is clear that an additional wear surface is introduced, thus increasing the area of articulation. Initial studies of these joints have shown good success, with 98% survival after 12 years by one account.[240] Other studies have shown these designs susceptible to the same delamination and abrasive mechanisms that affect less conforming designs.[241] Some studies have shown even higher wear rates than less conforming designs.[242]

In addition to analyzing worn surfaces, researchers retrieve and analyze wear debris. They determine the size distribution and shape of the retrieved particles. The biological activity of these particles has been studied as a function of size and morphology.[243] Studies of this kind have found that many of the particles surrounding THA are less than 1 μm long.[244] Larger debris has been reported in the tissue surrounding TKA than THA.[245] Although this may reflect the relative importance of delamination versus adhesive and abrasive wear in the two articulations, it may also reflect differences in lymphatic drainage from the two joints. The particle distribution is important because the biological activity of wear particles may depend on their size and shape. Thus certain wear processes may more likely lead to the generation of particles that cause osteolysis and aseptic prosthesis loosening.

Because of the multifactoral nature and long time scale of wear in replacement joints, it is difficult to understand prosthetic joint tribology through these means of clinical observation. Since TJA was only developed in the last 50 years, and wear processes may occur over the course of a decade or more, clinical observation has afforded the opportunity for few new TJA designs to be implemented and demonstrated as better than previous designs. In fact, it could be argued that no major improvement in TJA technology has been motivated by clinical observation since the change from PTFE to PE as an implant surface. Even the new, more conforming bearing designs have been difficult to assess in the absence of a complete understanding of the determinants of the tribology of TJA.

2.4.3 Knee and Hip Joint Simulators

As a result, there have been a number of laboratory approaches to simulating the tribology of TJA. The gold standard for prosthesis wear testing is the joint simulator. In a joint simulator, the joint replacement prosthesis is fixed to an apparatus that imitates the loading pattern and articulation in the replacement joint. In most modern simulators, the entire joint is sealed in a bag in which temperature-controlled bovine serum is circulated as a lubricant. Wear can be quantified gravimetrically by removing and weighing the tibial plateau or acetabular cup. Worn surfaces can be compared under microscopy to clinical retrievals. The wear debris generated can be collected and examined under scanning electron microscopy (SEM) for morphometric analysis. Both quantity and morphology of wear are validated by comparison with *in vivo* findings. Although hip simulators are more common, knee joint simulators are used extensively as well to test new prosthesis materials before and after being brought to market. Recommended parameters for this and other wear tests for metal-on-PE articulations have been established by the American Society for Testing and Materials (ASTM).[185]

Standard Methods for Hip and Knee Simulators

State-of-the-art hip simulators mimic motion in all three axes because the complexity of motion relates to the quantity and morphology of wear generated. Specifically, multidirectional motion has been shown to be both physiological and essential to the generation of wear particles of similar morphology to those found clinically.[246] Likewise, recent work in knee simulators has shown the importance of including rotation and anterior-posterior translation in laboratory articulations.[247] Current simulators employ three-dimensional articulation patterns with variable loads as

generated by biomechanical studies of human gait, such as that by Kadaba *et al.*[248] Most simulators replicate continuous walking or stair climbing at about one cycle per second.

Since both mechanical and chemical properties (elastic moduli, binding affinities, etc.) depend on temperature, the tribological behavior of the materials and the lubricant may be affected by temperature. Frictional heating necessitates temperature control in joint simulators; temperature is usually maintained at 37°C by the circulation of lubricant through a heat exchanger.

A number of studies suggest that temperatures in joint replacement may exceed 37°C. Finite element analyses have predicted temperatures of 42°C[249] and 60°C[250] in Co-Cr on PE THA, with even higher temperatures predicted for some ceramic on PE combinations; actual measurements *in vivo* have found between 35°C[250] and 43°C.[251] A hip simulator running at two cycles per second recorded subsurface PE temperatures of 70°C, apparently affecting PE wear rate.[252] Temperature increases brought about by frictional heating in simulator studies may affect tribology in an unpredictable and clinically irrelevant fashion.

For these reasons, the lubricant is typically used to control and monitor temperature during experimentation. This use of the lubricant typifies the consideration of lubricant in TJA; the only standards for lubricant treatment are that volume, concentration, and temperature be maintained.[185] Lubricant can be replaced or refreshed at the discretion of the researcher, and a number of different protocols have been used. The evolution of lubricant in laboratory wear tests is a relevant and lengthy topic, and is addressed separately (sections 2.4.6 and 2.4.7).

ASTM recommends that 0.2 to 0.3 percent sodium azide and 20 mM ethylene-diaminetetraacetic acid (EDTA) be added to the lubricant to discourage microbial growth and prevent calcium phosphate precipitation. If these are to be added, they should first be dissolved on 0.2 μm filter paper to remove particulate material.[185] The use of these additives is controversial, however, since they themselves could affect the tribology of the replacement joint. Table 2.4.1 summarizes the standard ASTM protocols for simulator experiments.

In addition to being very expensive to reproduce, complex articulations are hard to analyze quantitatively. Thus, simulator studies are largely empirical in nature. Consequently, it is desirable to conduct preliminary studies on the tribology of potential replacement joint couples prior to a simulator study. Joint simulators remain the gold standard as the final pre-clinical evaluation of the effects of long-term articulation of replacement joint prostheses, but studies of simpler geometries are appropriate to isolate specific mechanisms or articulating conditions.

2.4.4 Pin-on-Flat Apparatus

In a pin-on-flat (POF) device, a pin articulates on a flat disk in a cyclical fashion under normal load. The disk is situated in a cup filled with a temperature-controlled lubricant, which is typically bovine serum, as in simulators. Pins are removed periodically to be cleaned and weighed to determine material wear. The POF test simplifies the articulation between replacement joint surfaces to facilitate quantitative analysis while adequately approximating the important features of the articulation. Since

these devices are much less expensive than joint simulators, they are often used as a first step in evaluating the wear behavior of a new material for joint prostheses.

When approximating replacement joint articulations using *in vitro* experiments, one must balance the risks of missing relevant *in vivo* factors by oversimplifying the articulation and of making the experiment too complex to be useful analytically. Overarching this struggle is the cost associated with long and expensive experiments. Previous trial and error has taught us much about what parameters are necessary for laboratory testing. Some of these lessons have led to well-standardized protocols, and others are still being improved.

Standard Methods for Pin-on-Flat Apparatus

Those performing POF tests to simulate THA validate their apparatus by comparing wear morphology with clinical findings and by comparing their wear rates with the clinical wear factor, k, as discussed above in section 2.4.2. Initial POF tests resulted in wear rates on the order of 10^{-8} mm^3/Nm, two orders of magnitude lower than those found *in vivo*.[214,253,254] Replacing simple unidirectional motion with a rectangular motion track similar to that encountered in replacement hip joints[246,255] has resulted in wear rates and wear debris morphology more similar to that encountered in the replacement hip joint.[254] Although there have been some efforts made to simulate knee kinematics in a POF test,[227,256,257] no articulating motion has been standardized.

The period of a cycle in POF tests, like simulators, is typically one second, corresponding to an adult walking pace. The path of bi-directional motion varies, and is driven by the compromise between desiring to match *in vivo* conditions and desiring to speed up the experiments. The top speed of 30 mm/s in THA[248] gives a guideline for designing the perimeter tests. Both rectangular[258] and circular[254] shapes have been used, and both are considered acceptable, so long as the direction of motion changes relative to the PE surface.

Usually these tests employ a flat cylindrical pin on a flat disk. This enables easy calculation of a nominal contact stress that is constant throughout the test.[257] Others have used chamfered pins,[254] creating a variable nominal contact stress. For metal-on-PE articulations, the pin is invariably made of PE and the disk of metal even though a metal convex surface on a flat or concave PE would more accurately simulate TJA geometry. The choice of pin and disk minimizes gouging of the PE by a metal asperity bearing high load, and facilitates manufacturing of the metal surface. ASTM recommends that the surface roughness of polymers and metals be measured by a profilometer before experimentation.[185] Furthermore, to minimize the differences between *in vitro* conditions and *in vivo* conditions, it is customary to select a nominal contact stress similar to that found *in vivo*.

In choosing the size of the pins and the shape of their articulation, it is important to allow all areas of the counterface to be exposed to lubricant at some point during the cycle. This prevents lubricant starvation and the trapping of wear particles, which can alter wear rates artificially when pins that are too large are used.[13]

Means of data collection in POF have been standardized as well, to an extent. If wear rates are determined gravimetrically, pins are weighed on at least four occasions over the course of at least two million cycles.[185] At one cycle per second, a continuous test takes over three weeks to run two million cycles. Tests must be run for this long

because many researchers have seen periods of variable wear at the start of tests, followed by a constant steady-state wear rate. The reasons for such variation are debated.

Lubricant treatment in POF tests is similar to that in simulator tests. Due to differences in geometry and perhaps loading patterns, PE components in POF devices sometimes absorb lubricant during wear tests. In order to account for absorption, it is recommended that a control pin be soaked in lubricant under the same loading conditions as the pins tested.[185] Similar recommendations are made for joint simulators. Standard protocols used in POF tests are shown in Table 2.4.1.

Table 2.4.1 Standard methods used in laboratory experiments on the tribology of TJA These methods have been standardized, either by ASTM or by common practice. The methods are discussed in detail in the text. NaN_3 is sodium azide.

	Joint Simulator	*POF Apparatus*	*Friction Apparatus*
Motion	3-axis as determined by biomechanics	Bidirectional	Unidirectional
Temperature	37°C	37°C	No standard
Lubricant	Bovine serum or equivalent	Bovine serum or equivalent	No standard
Lubricant Additives	NaN_3 0.2 – 0.3% EDTA 20 mM	NaN_3 0.2-0.3% EDTA 20 mM	No standard
Clinical Standard	$k \sim 10^{-6}$ m^3/Nm debris morphology	$k \sim 10^{-6}$ m^3/Nm debris morphology	μ
Frequency	1 – 2 Hz	1 Hz	N/A
Speed	Determined by joint kinematics	20 – 40 mm/sec	Variable
Geometry	TKA or TJA	Cylindrical pin on flat metal disk	Flat or spherical pin on flat metal disk
Stress	Determined by biomechanics	Up to 40 MPa knee Up to 15 MPa hip	Up to 40 MPa knee Up to 15 MPa hip
Measurements	At least 4 over at least 2 Mcycles	At least 4 over at least 2 Mcycles	No standard
Calibration	Account for creep	Account for lubricant absorption	No standard

The relatively simple articulation created by the POF device does not fully match the complex stress patterns created by the geometry of the replacement joint articulation. For example, compressive, shear, and tensile stresses have been found in TKA by finite element modeling;[216] these are obviously not matched in a POF device. Nonetheless, as the mechanisms of wear have become better understood in TJA, the POF test may become increasingly valuable as a means of screening potential replacement joint couples.

2.4.5 Friction between Replacement Joint Couples

Wear tests require long time periods and are subject to wide variability, even when parameters are well-controlled. A more rapid tribological assay is a friction measurement. The wear test is more directly useful than the friction test, since the wear

particle causes the untoward biological response leading to loosening. Nonetheless, friction measurements can be useful because friction typically correlates with wear for a given articulating pair. Frictional work is the work done in articulation; this work changes the materials by plastic deformation, plowing, adhesion, and subsurface crack nucleation and propagation; these energy dissipation processes lead to surface damage. Thus, increased friction implies increased wear.

Choosing prosthesis materials solely based upon friction measurements can be misleading, as evidenced in the failure of the first Charnley prostheses. In this case, however, the comparison of μ was among different articulating pairs; the mechanisms of wear, and therefore the relationship between friction and wear, differed because the wear surfaces differed. This difficulty can be obviated by using identical wear surfaces (*i.e.*, PE), or by understanding and holding constant the material properties that determine wear particle generation.

In his unified theory of wear for PE, Wang positively correlated friction and wear for a metal-on-PE articulation. These results were supported by hip simulator experiments.[258] In these experiments, he altered μ by changing the radial clearance of femoral heads. Since he changed the geometry of articulation, other affected parameters besides friction may have altered wear rate. Therefore, the relationship between these factors may have been incidental.

Nonetheless, for a given articulating couple, such as metal-on-PE, it is reasonable to expect a positive relationship between friction and wear. In particular, when mixed or boundary lubrication takes place, a reduced coefficient of friction suggests less energy generated in articulation. Since there is less energy to be converted to adhesion, plowing, and plastic deformation, wear is likely to be reduced (though the relationship may not be linear). Therefore, friction apparatus may be ideal for determining the effects of lubricant: since the articulating couple can be preserved, small but significant differences in μ may be observed that would be obscured by the wide variability and long iteration times required for POF tests.

A number of researchers have measured μ to estimate lubrication in hip simulators. In one of the earliest THA simulations, Weightman *et al.* measured μ between 0.05 and 0.1 in three different metal-on-PE prosthetic hip designs lubricated by bovine serum.[259] In more recent work, Wang measured the torque generated in a hip simulator, and converted that value to a coefficient of friction, finding a range of 0.05 to 0.11 using a Co-Cr on PE THA lubricated by bovine serum. The range of μ was obtained by adjusting the clearance between head and cup.[258,260] The disadvantage of data taken from these sources is that joint simulators employ complex torques and loading patterns. The "coefficient of friction" determined from these tests was a ratio of average torques that may not adequately reflect the tribology of the articulation. Simpler motions are easier to understand analytically.

A number of POF-like devices have been used to measure friction between joint surfaces, though they have primarily imitated synovial joints rather than replacement joints. Such devices typically employ two loaded parallel surfaces in which one is moved at a fixed rate relative to the other, and the force between them measured. These tests are only a few minutes in duration, and so measure friction only, and not wear. By varying the normal load and the relative velocity of the surfaces, such devices can be used to

create a Stribeck curve for a given couple and lubricant. Apparatus of this kind have been used to measure the friction of cartilage-on-cartilage,[24,40] cartilage-on-glass,[40] cartilage-on-metal,[24] and latex-on-glass[44,46,261-263] as approximations of natural joint articulation.

The first report of a coefficient of friction in Co-Cr on PE came from Walker in 1973. He reported μ of 0.05 for synovial fluid-lubricating Co-Cr on PE,[64] though he didn't describe the device he used or the method of measurement. In 1979, Davis *et al* used a POF type apparatus to measure friction in replacement joint couples. They found that bovine synovial fluid reduced μ of PE on PE relative to saline, but only from 0.30 to 0.29.[40] In a number of other synthetic articulations, μ was actually increased when lubricated by synovial fluid rather than saline. None of these were metal-on-PE, however.

Others have followed Davis' lead in using such devices to estimate the tribology of replacement joint articulations. In 1998, Sawae *et al.* reported coefficient of friction between PE and both alumina and stainless steel lubricated by saline, bovine serum, and albumin solution. At the start of the tests, saline and water gave similar coefficients of friction (~ 0.05) for metal-on-PE. Eventually, μ increased to between 0.1 and 0.2 in the saline lubricated case, presumably due to the effects of a transfer film.[264] In 2001, Widmer *et al.* used a rotating POF device to measure the effect of plasma treatment on the friction between alumina and PE under lubrication by albumin solution.[265] In short experiments, this apparatus cannot directly measure wear in these surfaces, but friction measurements under boundary lubricated conditions can be used to estimate the efficacy of boundary lubrication.

Typical Methods of Friction Tests

There are no standards set for friction apparatus. ASTM does not comment on these tests as precursors to clinical trials of replacement joint materials, though they are an obvious antecedent to POF tests. Thus, common methods are described with reasoning for their use (Table 2.4.1), rather than references to standard protocols. Although many parameters in friction tests are similar to those in POF tests, some parameters differ. Velocity in friction tests may be continuously controlled. It is important that velocity be set at a speed that is relevant *in vivo*. Bi-directional motion is not necessary in friction tests, and is rarely employed, since it makes μ more difficult to calculate. Since the tests are brief, the geometry of the pins is not altered during the test. Thus, spherical pins can be used, so as to enable convenient calculation of Hertzian contact stresses (rather than nominal contact stresses). There is no standard at all with respect to lubricants in friction tests, and tests are often run at room temperature to minimize the complexity of the device. Due to the brevity of tests, more data can be generated in a short time using friction tests than wear tests.

2.4.6 Wear Testing – from Dry to Water to Bovine Serum

Lubricants serve a variety of roles depending on the nature of the articulation. Some common roles of lubricants include:[208,266] (1) preventing particle agglomeration at the interface; (2) removing particles from the interface; (3) preventing adhesion between the surfaces; (4) dispersing thermal energy from the surfaces; and (5) shielding the

surface from plowing and plastic deformation. In a given application, some of these roles are more relevant than others.

Originally, PTFE and then PE were chosen because of excellent μ in the absence of lubricant. Thus, the roles of joint fluid in lubricating TJA were ignored. The simulator and POF tests described above were primarily aimed at testing potential materials for use in joint replacements, not at testing intrinsic patient factors such as joint fluid. Nonetheless, within the backdrop of these experiments, our understanding of the role of the lubricant in the tribology of TJA has evolved. Initial experiments on the tribology of metal-on-PE compared wet and dry conditions, using distilled water as a lubricant (*e.g.*, Dowson and Harding[267]). Since dry articulation generated ten times as much wear as lubricated articulation, it was not possible to completely ignore the effect of lubricant. Of the roles of lubricant listed above, distilled water most likely contributed to 2 and 4. Particle agglomeration (1) has not been observed in replacement joint articulations, though wear by third bodies has been observed. Protecting from plastic deformation and plowing (5) are roles of fluid film and boundary lubricants. Water may perform a boundary lubrication function in this articulation, but it is not generally considered a good boundary lubricant in other articulations.

In 1978, McKellop *et al.*, noted that using bovine serum instead of distilled water in a unidirectional POF apparatus resulted in lower wear rates and wear morphology less dissimilar from those found at retrieval.[214] In particular, they noted that grossly visible PE transfer films formed repeatedly on the metal counterface when lubricated by distilled water, but formed less often when lubricated in serum. This finding was very important because it cast doubt on the ability to rank how materials would perform in vivo by their performance in water-lubricated wear tests.

Bovine serum appeared to reduce adhesion (3) relative to distilled water; it is possible, however, that adhesive wear still occurred under serum lubrication, if a component of serum facilitated removal of adherent particles before they were grossly visible. Transfer films have not been observed clinically, so joint fluid apparently prevents (or at least removes) adhesive wear particles *in vivo*. Furthermore, a boundary lubrication role (5) may be performed by one or more components of bovine serum not present in distilled water. This component may or may not be present and active in joint fluid. Although it was not clear whether bovine serum was a good choice to simulate joint fluid, it was clearly an improvement over water.

The work of McKellop *et al.* was not the first to evaluate bovine serum, but it may have been the first to make a clear recommendation for its use. They state, "In view of these results, we have concluded that wear tests of materials for prosthetic joints should be conducted with blood serum as a lubricant in insure that the wear processes adequately simulate those occurring *in vivo*."[214] Bovine serum was a rational step forward from distilled water in that it better approximates joint fluid in content. Immediately after surgery, blood fills the synovial cavity; when the synovial membrane repairs, the joint fluid filling the cavity is primarily blood dialysate, as synovial fluid is. Therefore, a serum-based lubricant diluted to contain roughly the amount of protein expected in synovial fluid is likely to lubricate more like joint fluid than distilled water would.

Since McKellop's preliminary work, many others reported improved results with bovine serum versus distilled water. In 1993, Cooper *et al.* reported decreased transfer

films using a "protein-containing" lubricant.[253] In 1994, Derbyshire *et al.* reported much lower wear rates in a POF tester using bovine serum rather than distilled water.[268] In 1996, Good *et al.* reported greater repeatability using bovine serum rather than distilled water in a hip simulator for the articulation of PTFE on Co-Cr.[269] In 1996, Wang *et al.* reported wear particles in a hip simulator more similar to those found *in vivo* when lubricated by bovine serum compared with distilled water.[270, 1996] In 1999, Besong *et al.* showed similar results in POF tests, and found fourteen times as much wear in distilled water.[271]

As a counterpoint, Sawae *et al.* reported in 1998 no volumetric difference in wear comparing saline and bovine serum as lubricants of stainless steel on PE in a POF apparatus.[264] Friction was also the same between the couples at the start of articulation. These tests were conducted at 3 MPa nominal contact stress; it is possible that the lubrication functions performed by bovine serum but not distilled water are not necessary at low contact stresses, thus resulting in similar wear rates and friction. On the other hand, wear morphology was quite different between the two cases, suggesting that different wear processes were at work.

Despite a plethora of rational and empirical evidence, it took many years for the improvement in lubricant from water to serum to become widely accepted. The slow progress was due, in part, to the cost and time required to run wear tests, which dissuaded researchers from employing new protocols until they had been well-accepted. A conservative approach was additionally encouraged by the many relevant parameters being researched simultaneously. For any given study, the most well-established protocol was typically used with regard to all parameters *not* being studied (including the lubricant). This enabled easier comparison among laboratories. Even now, distilled water tests are typically run as a standard to compare against bovine serum lubrication.

Currently, bovine serum is firmly established as the standard lubricant for laboratory wear tests of replacement joint materials, though there is some debate about appropriate dilution. The ASTM standard tests calls for bovine serum in distilled water (diluted up to 75% by volume at the discretion of the researcher). An alternative lubricant should be used "only when it can be shown that the lubricant reproduces clinical wear mechanisms as well or better than bovine serum."[185]

Bovine serum is clearly an improvement over distilled water. It is, however, an inadequate approximation of joint fluid for use in tribological experiments of replacement joint articulations. Aside from the likely differences between bovine serum and joint fluid, bovine serum does not appear to be biologically stable. Bell *et al.* showed that lipid content of bovine serum changes with time, even within the time frame of a wear test;[272] similar findings have been reported in protein content.[273] As discussed in section 2.2, proteins and phospholipids may provide boundary lubrication in metal-on-PE articulations. Therefore, it is not even clear what components of *bovine serum* are relevant to lubrication of replacement joint articulations. Ironically, even in the first recommendations for the use of bovine serum, McKellop *et al.* note that "lubrication with synovial fluid might provide an even closer replication of the physiological environment."[214] It is my intention to work toward replacing bovine serum as a lubricant in laboratory wear tests. This is one of the main purposes of determining the content of joint fluid (Chapter 4) and determining how these components affect friction between replacement joint materials (Chapter 5).

2.4.7 Beyond Bovine Serum – Physiological Lubricants

The first mention of a synthetic fluid to match synovial fluid came in 1971,[274] though not in the context of TJA wear tests. It was not until recently, however, that a few researchers have been publishing tribological experiments on replacement joint couples in synthetic lubricants that attempt to imitate joint fluid. Three groups have used individual components of joint fluid in lieu of bovine serum as a lubricant in POF tests. A fourth group has conducted friction experiments with lubricants composed of multiple constituents of joint fluid.

In 1997, Ahlroos and Saikko compared wear morphology of PE on stainless steel in bovine serum, soy protein, soy lecithin (a phospholipid), and a sample of replacement joint fluid using a unidirectional POF test and a hip simulator. In these tests, soy protein was the only lubricant that prevented a transfer film from forming on the stainless steel counterface.[275] In similar experiments, they reported the performance of DPPC in water and in saline as much improved over soy lecithin.[276] These tests were unidirectional, however, and, as discussed above, it has since been reported (even by these authors)[254] that bi-directional motion is a necessary parameter in TJA wear experiments. The above experiments did not replicate wear morphology encountered *in vivo*.

Ahlroos and Saikko then began experimenting with a bi-directional POF device.[277] They found that DPPC reduced wear to near zero despite the formation of a transfer film. Under the same loading conditions, bovine serum resulted in wear rates similar to those found in hip simulators. Based upon these results, the authors dismissed DPPC as a possible boundary lubricant *in vivo*, since, if it were active, it would prevent wear in TJA altogether. They did not comment on the importance of the near zero wear or the transfer film.

In 2000, the same authors compared bovine serum to albumin and γ-globulin as lubricants in their bi-directional POF device. They used 70.7 N loads and 8.9 mm PE wear faces against stainless steel. Initial wear rates were higher for bovine serum than for either protein. At steady state, they found the proteins to result in similar wear rates and coefficient of friction as bovine serum. This result does not demonstrate that these proteins are responsible for the tribology of bovine serum in this articulation.[278]

A second group examining physiological lubricants is Sawae *et al.* In 1998, they reported the effect of certain synovial fluid constituents on friction and wear patterns of PE on both metal and ceramic counterfaces.[264] In particular, they used physiological concentrations of HA and albumin in saline and in distilled water as lubricants. They found lower friction with HA as a lubricant, and found different patterns of wear with albumin versus bovine serum. Like Saikko and Ahlroos' first experiments, these tests were unidirectional, and obtained wear factors consistent with other unidirectional tests (10^{-8} mm^3/Nm), implying different wear mechanisms than are found *in vivo*. Furthermore, they used HA of low molecular weight, making a less viscous and viscoelastic lubricant than joint fluid. Finally, they used grooved pins to try to prevent a hydrodynamic film from forming. The unusual surface geometry used may have affected lubrication and wear mechanisms, making their results difficult to compare with others'.

The third group, Bell *et al.*, argues that bovine serum is not sufficiently stable for wear tests, and has sought an alternative lubricant. In 2000, Bell reported unidirectional PE on stainless steel POF experiments comparing bovine serum to another protein-based

lubricant; wear rates were similar to those obtained with serum, but wear morphology was different, so the lubricant was rejected.[272] In 2001, she added small amounts of phosphatidylcholine (0.5, 5.0, and 50 mg/ml) to this protein-based lubricant and lubricated metal-on-PE in a hip simulator. She reported that the lubricant with phospholipid reduced wear threefold relative to bovine serum, even at the lowest concentrations.[62]

Finally, Kitano *et al.* have produced steady-shear viscosity curves and μ versus angular velocity curves for PE on stainless steel using a variety of lubricants. The lubricants tested included buffered HA as well as buffered HA with albumin, γ-globulin, or DPPC added. These were tested through a range of pH.[184] The authors found evidence of a mixed lubrication regime and a fluid-film regime. Friction in both regimes depended somewhat on pH. The normal stress in these tests was 12.5 kPa, which is quite lower than physiological stresses. Although a number of lubricants similar to joint fluid in formulation were used, no comparison was made between these fluids at physiological pH.

There is lacking a systematic analysis of the tribology of metal-on-PE comparing lubricants formulated within the range of joint fluid compositions. This has been impossible, since the range of joint fluid content has not been known, and is first reported in Chapter 4 of this thesis. Using the composition determined in Chapter 4, this comparison is made in Chapter 5.

2.5 The Content of the Thesis

The body of this thesis consists of four independent studies investigating joint fluid and its role in the tribology of TJA. Chapter 3 examines the rheology of joint fluid from TKA patients, reporting on gaps in data as described in section 2.3. The rheological properties of joint fluid relate directly to fluid film lubrication in TJA, as discussed in section 2.1. Chapter 4 examines the composition of joint fluid from TKA patients. In particular, protein, HA, and phospholipid content are determined, as discussed in section 2.2. These data, not previously reported in a comprehensive manner, relate to both boundary and fluid film lubrication as discussed in section 2.1. Chapter 5 evaluates the effects of various components of joint fluid on the friction of PE-on-metal articulations. Chapter 6 departs slightly from an analysis of the effect of lubricant on TJA tribology, in that it examines, through a POF device, the effects of contact stress and area on the wear rate of PE on Co-Cr within a physiological range. Chapter 6 also provides an empirical link between friction and wear for PE-on-metal. These two chapters are unified by a conceptual model through which the role of various parameters in TJA tribology is understood. Finally, Chapter 7 summarizes the work that has been conducted, discusses the practical application of this work to TJA, and makes recommendations for further study.

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CHAPTER 3

RHEOLOGY OF JOINT FLUID

While the properties of joint fluid may affect the tribology of joint replacement prostheses, the flow parameters of joint fluid have not yet been examined in the context of TJA. The objective of this study was to evaluate the flow properties of joint fluids in patients undergoing index TKA or revision TKA, and of other relevant fluids. It was hypothesized that there would be an alteration of the properties of joint fluid resulting from TKA. The steady-shear viscosity and storage and loss moduli were evaluated in joint fluid from fifty-three arthritis patients undergoing TKA, fourteen patients undergoing revision of a previous TKA, and five patients presenting with joint effusion after TKA, among other patients. The steady-shear viscosity varied over three orders of magnitude among samples obtained from patients undergoing TKA, spanning "normal" and "diseased" ranges established previously. Fluid obtained at index TKA was more viscous than fluid obtained at revision TKA as demonstrated using several statistical methods, though the more significant finding was the wide variability in both groups. Both groups exhibited degenerate flow properties when compared to synovial fluid from healthy individuals. Further examination of the connection between flow properties and the tribology of joint replacement prostheses is warranted.

3.1 Introduction and Objectives

As discussed in Chapter 2, both boundary and fluid-film lubrication likely contribute to the tribology of the prosthetic joint as they do in the natural joint,[1-4] though the relative contributions of each type of lubrication may differ. Despite the importance of tribology to the function of joint prostheses, very little has been reported regarding the mechanisms of lubrication in these articulations. In the context of fluid-film lubrication, joint fluid flow properties determine tribology. Flow properties of synovial fluid vary substantially among patients with normal and diseased joints (see section 2.3). The variability in joint fluid properties after TJA could thus contribute to the widely varying wear rates and clinical outcomes found *in vivo*.

Understanding the role of joint fluid in fluid film lubrication requires an assessment of its bulk fluid properties. It must be shown that the variability of synovial fluid flow properties found among knees in the general population exists in patients undergoing TKA and persists in joint fluid after TKA. In particular, the steady-shear viscosity and linear viscoelastic properties are flow parameters that can be used to characterize a joint fluid sample's contribution to fluid film lubrication in TKA, as they do in the natural knee. Both steady-shear viscosity (Table 2.3.1) and linear viscoelastic properties (Table 2.3.2) have been examined previously in both normal and diseased knees. There are relatively few data, however, evaluating these features of joint fluid in arthroplasty patients.

3.1.1 Specific Aims and Hypotheses

The objective of this study was to evaluate the flow properties of joint fluid in the context of TKA. This study was motivated by the need to understand the role of joint fluid in the lubrication of TJA. Joint fluid from knees was chosen over joint fluid from hips because more fluid can usually be obtained from the former. Two hypotheses were tested. First, it was hypothesized that flow properties vary widely in the joint fluid obtained from TKA patients at revision surgery. This hypothesis is essential to the thesis: if verified, would support a connection between variability in joint fluid flow properties and wear in TKA; if joint fluid properties are relatively constant, variability in joint fluid flow properties do not explain variable tribology and clinical outcome in TJA. A second hypothesis was that the flow properties of joint fluid obtained at revision TKA would differ from that of synovial fluid from OA patients obtained at TKA. This second hypothesis is less crucial to the thesis than the first, but it provides some indication whether previous analysis of synovial fluid can be applied to analysis of joint fluid in TJA. OA patients undergoing TKA are a control group, and are used to compare the present work against previous reports. A related aim was to compare the properties of these fluids to those that are or could be used for laboratory wear testing of joint prostheses.

The steady shear viscosity and linear viscoelastic properties were evaluated in joint fluid from patients undergoing TKA and patients undergoing revision TKA. These rheological properties were compared to those previously reported in normal and diseased patients. The rheological properties of bovine serum currently used in knee simulators and wear testing were evaluated and compared with the properties of joint

fluid. Finally, the flow properties of two commercially available hyaluronic acid preparations were evaluated.

3.2 Materials

Seventy-nine synovial fluid samples were obtained from OA patients during TKA surgery. Twenty joint fluid samples were obtained during revision of aseptic TKA in other patients. Eight synovial fluid samples were aspirated from natural joints with effusions. Three fluid samples were aspirated from effused joints that had previously undergone TKA. Finally, one sample was obtained during revision of a unicompartmental knee arthroplasty. All samples came from Baw Beese Sports Medicine and Joint Care, Brigham and Women's Hospital, New England Baptist Hospital, or Massachusetts General Hospital in accordance with a protocol approved by each hospital's respective Institutional Review Board. All samples were inspected upon receipt, and described as non-inflammatory, inflammatory, septic, hemarthrotic, or bloody, in accordance with clinical characterization of synovial fluid aspirates.[5] Twenty-six synovial fluid samples from TKA, six samples from revision surgery, and three samples from effusion were not used because there was insufficient fluid for mechanical testing. Patients ranged from 37 to 89 years old, with an average age of 68 years. Of the fourteen joint fluids from revision TKA whose properties were successfully measured, eight had undergone revision because of wear-related osteolysis and six because of mechanical problems not specifically related to wear. Patient information was obtained from medical records. See Appendix A for patient summaries.

The standard lubricant employed for laboratory wear testing, bovine serum, was also tested in this study. All bovine serum samples came from Life Technologies calf serum lot number 1023609, with 73 mg/ml total protein, diluted to 40% by volume in distilled water. Additionally, flow properties were measured for two commercially available hyaluronic acid preparations, Supartz (Smith & Nephew, Memphis, TN) and Orthovisc (Anika Therapeutics, Woburn, MA), employed as injectable agents for the treatment of osteoarthritis. All Supartz samples came from Artz lot number 9Z683A 2002.11, and contained 10 mg/ml sodium hyaluronate at molecular weight 0.62 to 1.17 MDa. All Orthovisc samples came from Anika Therapeutics lot number 60382000, and contained 1.4 mg/ml sodium hyaluronate at mean molecular weight 1.39 MDa. Because the flow properties could be measured repeatedly within ten percent, it was not necessary to test more than three samples of each fluid.

3.3 Methods

3.3.1 Evaluation of Steady-Shear Viscosity

The flow parameters of each sample were evaluated on a CSL 500 controlled stress rheometer (TA Instruments, New Castle, DE). The rheometer was first calibrated with Cannon Certified Viscosity Standard mineral oil using a range of imposed stress from 0.1 Pa to 10 Pa or more. Flow properties were evaluated on 2.5 ml of each sample using the double cylinder Couette flow geometry or cone and plate geometry of radius 3 cm and cone angle 1°.

At the start of each test, the sample was sheared at 500 s^{-1} for ten seconds to minimize the effect of past shear history. In order to evaluate the steady shear viscosity as a function of shear rate, a given shear stress was initially applied, and the steady-state shear rate measured. The shear rate resulting from an imposed shear stress was determined using a stepped ramp sweep decreasing logarithmically over two decades of shear stress. For each of ten steps in the first decade, the mean shear rate was measured over twenty second intervals until the measured mean shear rates within two consecutive intervals agreed to within one percent. For each step in the second decade, the mean shear rate was measured over forty seconds until two consecutive intervals agreed to within three percent. In some cases, the mean shear rate at a given step was measured over only one interval of eighty seconds. The measurements continued in this fashion until reaching the minimum deformation rate measurable on the rheometer. Typically, it was possible to evaluate the deformation rate over 1.5 to 2.5 decades of shear stress for each joint fluid sample. Steady shear viscosity could be measured in sodium hyaluronate samples over three orders of magnitude of shear stress.

To compare data from different samples, the viscometric data were fitted to a simplified Cross viscosity model.[6] In this model, the shear rate $\dot{\gamma}$ and viscosity η are related by the function $\eta = \eta_0/(1+(c\cdot\dot{\gamma})^d)$ (*Equation 2.3.1*), where η_0 is the viscosity at low shear rate; c is the consistency, which is related to the longest relaxation time of the fluid; and d is the rate index, a dimensionless variable that characterizes the negative slope on a double logarithmic plot of the shear-thinning region, in which $\eta \sim \dot{\gamma}^{-d}$. The data were fit to the simplified Cross model using an iterative chi-squared minimization method on the natural logarithm of the shear rate and viscosity using Igor Pro software (WaveMetrics Inc., Lake Oswego, OR). A second method of comparison, the viscosity at 1 Pa shear stress (η_{1Pa}), was also used as a comparative tool among samples.

3.3.2 Evaluation of Linear Viscoelastic Parameters

A small amplitude oscillatory shear stress test was performed on each sample to measure the linear viscoelasticity of joint fluids. During each test, the strain response to a small, sinusoidal shear stress was measured for twenty-five frequencies between 25 Hz and 0.1 Hz, inclusive. For sufficiently small strains, the output is a sine wave of different phase and amplitude than the input. The portion of the strain in phase with the stress input is related to the elastic character of the fluid sample, and is expressed as the storage modulus, G'. The portion of the strain out of phase with stress is related to the viscous character of the fluid sample, and is expressed as a loss modulus, G'' or dynamic viscosity, $\eta' = G''/2\pi f$.[7] These parameters describe the relative importance of elasticity and viscosity in small amplitude oscillatory motion. Viscoelastic properties were measured for five different torque (shear stress) inputs: 25, 50, 100, 200, and 300 μNm. Since the fluid response is linear for small deformations, single plots of the linear storage and loss moduli as functions of frequency were compiled from these curves. To compare differences between samples, the viscoelastic crossover frequency f_c and modulus at crossover $G_c = G'(f_c) = G''(f_c)$ were calculated when possible.

Since crossover did not always occur within the range of frequencies measured, especially in fluids with smaller moduli, other parameters were used to compare samples.

In particular, the moduli at 0.5, 2.5, and 5.0 Hz ($G'_{0.5Hz}$, $G''_{0.5Hz}$, etc.), were parameters used to compare samples. These parameters facilitated comparison to the work of others, who tabulated results at specific frequencies.

3.4 Determination of Methods

The apparatus and experimental methods used to determine the viscosity of joint fluid over a range of shear rates were chosen through an iterative method. After a repeatable and convenient protocol had been determined, efforts were made to validate the experimental protocol. Certain portions of the protocol determination were explained in a thesis previously submitted in partial fulfillment of a master of science degree.[8] These portions are summarized, but not repeated here.

3.4.1 Storage and Handling of Joint Fluid

The first samples were centrifuged at 1500 rpm at 18°C for ten minutes to separate cells and particles, based upon previously reported protocols.[9] This practice was soon deemed neither necessary nor useful, as particles were best separated through careful pipette technique. Samples were refrigerated between aspiration and evaluation when short-term storage was necessary (*i.e.*, a few days). Despite evidence that the properties of joint fluid are not affected by long-term refrigeration,[10] samples were stored at -70°C when long term storage was necessary.

When testing schedules required transport, no temperature control mechanism was used while the samples were in transit. This necessarily entailed exposing the samples, with limited insulation, to outside air temperature for ten to fifteen minutes. Although the effects of freeze/thaw cycles on the flow properties of synovial fluid appear to be small,[11] an effort was made to minimize the number of times a sample was thawed.

During transport and shipping, joint fluid samples were treated like other biological specimens. The sample was held within an airtight container packed in absorbent material within a second airtight container. This outer container was labeled with a bright orange biohazard label. In case of breakage of the inner container, the second container protected against leakage, in accordance with the guidelines of both the U.S. Department of Transportation and the Occupational Safety & Health Administration.

3.4.2 Experimental Apparatus

Initial estimates of joint fluid viscosity based on prior work and preliminary experiments suggested that a Couette cell, rather than cone-and-plate geometry, was appropriate for joint fluid. The CSL 500 rheometer used allows for Couette double cylinder geometry, and was available to be devoted to experimentation on biological fluids. Although other experimental instruments could have been used to measure joint fluid properties under different conditions, these conditions matched what has been consistently reported in the literature, and so fit most directly into the context of the findings of others (cf. section 2.3.1).

One limitation of this apparatus is that the Couette cell used measures shear rate at a fixed gap of 300 μm. In replacement joints, the gap between surfaces varies, but it is likely that relevant tribology occurs at substantially smaller gaps (less than 1 μm).

Although it has not been shown that the properties of the fluid differ when examined on a smaller scale, findings in other fluids[12,13] and the previous discussion of the macromolecules of synovial fluid (cf. Section 2.2) suggest that they may. Nonetheless, in order to compare with the work of others in similar fluids and in order to reduce experimental complexity, use of a standard 300 μm gap was an appropriate starting point for characterizing joint fluid.

A second limitation is that the Couette cell cannot measure normal stress differences. At some point, it may be beneficial to examine the normal stress differences generated by these fluids when under shear, in particular to compare with that found in healthy synovial fluid (cf. Section 2.3.4), but that lies outside the scope of this thesis.

3.4.3 Calibration

Initially, the rheometer was calibrated using Canon certified viscosity standard with $\eta = 3.417$ mPa·s at 25°C. After evaluating the viscosity of several samples, it became clear that most samples were substantially more viscous than this standard, particularly at low shear rates. Canon certified viscosity standard S60, with $\eta = 101.5$ mPa·s at 25°C, was chosen to replace it. In performing a calibration, the standard fluid of known viscosity was measured using a continuous shear stress ramp decreasing logarithmically from 45 Pa to 0.1 Pa over the course of 150 seconds. A sample calibration curve is given below in Fig. 3.4.1.

As demonstrated by the linear curve in Fig 3.4.1, both the expected and measured curves followed a Newtonian relationship (*i.e.*, shear rate linearly related to shear stress). The absolute deviation from the expected value increased as shear stress increased. On the other hand, the deviation as a percentage of shear rate was larger at low shear rates, as reflected in the logarithmic plot in Fig 3.4.1. This result demonstrated that error increased as stress was reduced below the lower limit of shear stress for the CSL 500 set by the manufacturer (0.5 Pa for Couette double cylinder geometry).

In this case, the measured shear rate was lower than the expected shear rate at all shear stresses by a factor of approximately 1.26. Stated another way, when fitting the expected and measured curves to a Newtonian model, the measured viscosity was 1.26 times the expected viscosity. Since a 25% error was not acceptable, and since this error was both irreducible and reproducible, it was corrected for in data analysis.

Consequently, a calibration was performed each day in which testing was to be conducted. A calibration constant C was determined using the equation

$$C = \frac{\dot{\gamma}_{Expected}}{\dot{\gamma}_{Measured}} = \frac{\tau/\eta_{Expected}}{\dot{\gamma}_{Measured}} = \frac{\tau/\dot{\gamma}_{Measured}}{\eta_{Expected}} = \frac{\tau/\dot{\gamma}_{Measured}}{101.5\,m\,Pas}, \qquad \textit{Equation 3.4.1}$$

where $\tau/\dot{\gamma}_{Measured}$ was a constant obtained by fitting the measured shear rate versus shear stress curve to a Newtonian viscosity model using the method of least squares on TA Data software (TA Instruments, New Castle, DE). Each shear rate measured for a sample in subsequent experiments was multiplied by C to find the actual shear rate using the formula $\dot{\gamma}_{Actual} = \dot{\gamma}_{Measured} \times C$. This method not only checked the accuracy of the rheometer, but accounted for measurement bias. The reliability of this method of calibration depended on the repeatability of measurement and a similarity between the

standard viscosity and that of the sample. In most cases, C fell between 0.8 and 1.2 using this method. The example given in Fig 3.4.1 shows the largest deviation from $C = 1.0$ obtained. Further discussion of calibrating the Couette cell is given in Appendix B.

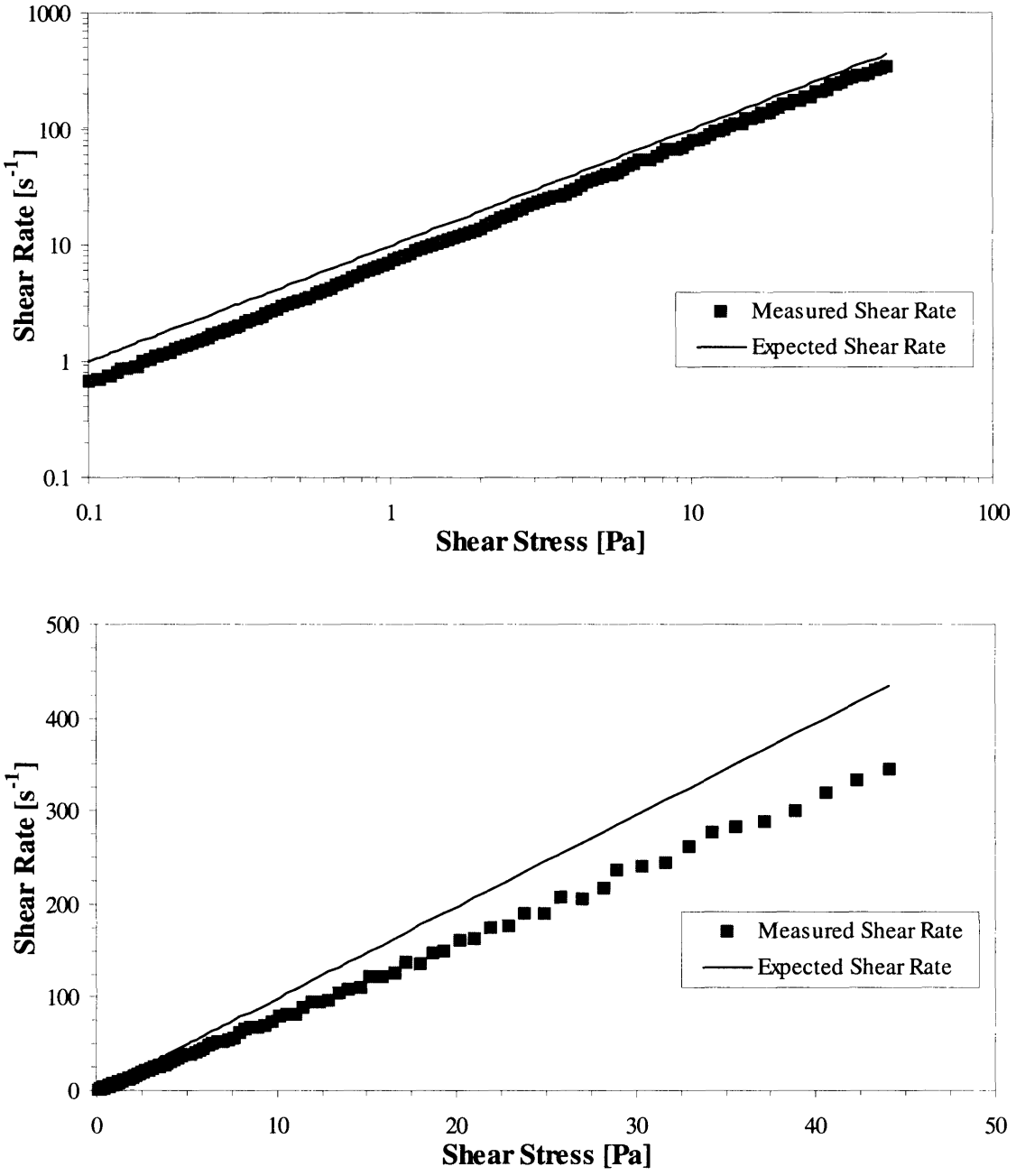

Fig 3.4.1 Comparison of measured shear rate of Canon certified viscosity standard S60 to its expected value The closed squares are measurements, and the line represents the expected curve. The top figure is shown on a log-log scale, whereas the bottom figure is shown on a linear scale. The ratio between the expected and measured viscosity, as calculated by Equation 3.4.1, was 1.26. This calibration was the worst calibration curve obtained in the course of experimentation (as defined by the largest deviation from 1.0). This calibration was performed on March 21, 2002.

A number of possible sources of this offset in calibration were considered and eliminated. For example, temperature and bearing friction corrections were verified, and the rate of the shear stress ramp in calibration was slowed to demonstrate that it had no effect. The most likely source of the remaining error lay in misalignment of the Couette cell. This is evidenced by the fact that the calibration error could be minimized by slightly adjusting the Couette cell while shearing the standard oil. Due to many years of use, the Couette cell had become damaged, and this misalignment could not be corrected easily.

3.4.4 Shear Stress and Shear Rate Ranges

The CSL 500 with Couette double cylinder can measure shear rates as high as 1000 s^{-1} with a stated resolution of 2 x 10^{-3} s^{-1}. The manufacturer states limits on input torque that correspond to 20 to 0.5 Pa using the Couette double cylinder, though the software permits lower torque inputs. In determining η_0, it was necessary to use shear stresses as low as 0.1 Pa, below the lower limit proposed by TA Instruments for its device. This practice was sometimes necessary to ensure measurement of viscosity in the low-shear rate plateau region. This practice was validated using viscosity standard calibration, as discussed above, but, as discussed in Appendix B, the use of these data points was related to significant error outside the approved operating range of the equipment. Although it would be necessary to examine the properties of joint fluid outside this range in order to *fully* characterize this fluid, this range fit most accurately the range examined by others, and tended to capture both shear-thinning and the low shear rate plateau.

3.4.5 Temperature

In my M.S. thesis, I demonstrated that viscosity of joint fluid varies only slightly according to the Arrhenius model through the range 25°C to 40°C at 1 Pa shear stress.[8] Protein denaturation, aggregation, and precipitation all increase with temperature, so 25°C was a more preferable temperature at which to evaluate samples than 37°C, even though the latter would be more physiologic. This temperature was consistent with the work of others (cf. Section 2.3.7). One sample was evaluated at 25°C and 15°C (Study ID H23) and another at 25°C and 20°C (Study ID H17) to assess the effect of further lowering temperature on the viscous parameters of joint fluid.

3.4.6 Steady-Shear Viscosity Protocol

Since the CSL 500 is stress-controlled, direct control of stress was chosen for the steady-shear viscosity measurements. This afforded greater stability than use of a feedback mechanism to measure and control shear rate would have. A maximum shear rate of 500 s^{-1} was chosen based on the maximum angular velocity of the device, as discussed above. After the shear stress corresponding to this shear rate was determined, the sample was sheared at 500 s^{-1} for ten seconds to reduce the effects of shear history. Then shear rate was measured at a number of shear stresses between that corresponding to 500 s^{-1} and 1.0 Pa. The points measured in this "first decade" were all values of shear stress in the geometric series {1.00, 1.26, 1.59, 1.99, 2.51, 3.16, 3.98, 5.01, 6.31, 7.94, 10.0, ...} corresponding to a shear rate of 500 s^{-1} or less. This method ensured equal

logarithmic separation between all points, and ensured that viscosity at 1 Pa shear stress would be measured directly (without the need for interpolation). The twenty second interval was deemed sufficient to determine steady shear rates. Typically, the second and third intervals agreed within one percent to determine equilibrium, so each data point was generated in one minute.

For the range 1.0 Pa to 0.1 Pa, a similar ten point per decade geometric series was used. In order to obtain a steady shear rate within a sufficient time frame, the protocol was modified to measure mean shear rate over 40 seconds, and to accept variation within 3% as equilibrium. In some cases, viscosity appeared to increase slowly without end at low shear rates. Thus, this protocol did not always obtain a steady-shear viscosity. In early cases, it was necessary to end the test with limited data. In later cases, some of these samples were tested using a modified protocol to avoid thixotropic effects (cf. section 2.3.3). In these cases, apparent viscosity was measured in the second decade over 80 seconds without waiting for equilibrium. Furthermore, two samples were evaluated using the protocol of Oates *et al.*[14] to evaluate whether the thixotropic effects observed in synthetic synovial fluid could be measured in human samples.

3.4.7 Modeling

It was immediately evident that the data were too far spread to be compared directly. In particular, shear thinning, combined with the relative uncertainty of measuring η_0, made it necessary to fit the data to a model. The first model chosen was a Cross model with high shear rate and low shear rate plateaus using the method of least squares on TA Data software (TA Instruments, New Castle, DE). This model used a method of least squares and fit data to the function

$$\frac{\eta-\eta_\infty}{\eta_0-\eta_\infty}=\frac{1}{1+(c\dot{\gamma})^d}, \qquad \textit{Equation 3.4.2}$$

where η_0, η_∞, c, and d are constants. The significance of η_0, c, and d are identical to that from Equation 2.3.1 discussed in section 2.3 and above in section 3.3.1. The viscosity at high shear rate, η_∞, was included based upon limited experimental data available. Specifically, Cooke *et al.* plotted viscosity versus shear rate for two joint fluid samples from TJA, showing a concave curve that suggested an asymptotic lower limit for viscosity.[15] Equation 3.4.2 simplifies to Equation 2.3.1 at intermediate and low shear rate.

Initial data demonstrated no high-shear plateau (*e.g.*, Fig. 3.5.2), but the use of the model persisted because it was expected that the viscosity would not drop below that of water (1 mPa s), even at high shear rates. Eventually, the model was discarded because it was not robust. In particular, slight variations in high shear data greatly affected η_∞, which, in turn, substantially impacted d and, to a limited extent, the other parameters. Consequently, a new model was chosen, a Cross model that did not include a high-shear plateau. Data were fit to Equation 2.3.1 using IgorPro (WaveMetrics Inc., Lake Oswego, OR), as discussed in section 3.3. Specifically, the natural log of each shear rate ($\ln\dot{\gamma}$) and each viscosity ($\ln\eta$), after being calibrated as discussed above, were fit to the function

$$\ln\eta = \ln\eta_0 - \ln\left(1+\left(c*e^{(\ln\dot{\gamma})}\right)^d\right). \qquad \textit{Equation 3.4.3}$$

This model produced η_0, c, and d as parameters to meaningfully characterize the data, allowing for useful comparison among samples.

In addition, η_{1Pa} was used as a simple and direct means to compare among samples. This parameter was derived from direct measurement, rather than a parametric model.

3.4.8 Linear Viscoelasticity Protocol

Storage and loss moduli were chosen to compare among samples as a means to separate energy dissipation from energy storage. Since much of Balazs' work on viscoelasticity of synovial fluid employed these parameters (section 2.3.2), these made for obvious choices in the present work.

The range of frequencies used, 0.1 to 25 Hz, includes a range of frequencies encountered during motion *in vivo*, such as 1 Hz (walking) and 2.5 Hz (running). Although higher frequencies could be used to more completely characterize the fluid, they do not directly relate to frequencies encountered *in vivo*. Frequencies below the lower limit of measurement do occur *in vivo* in situations such as long periods of standing, in which the time scale is on the order of minutes. Previous work suggested that elastic behavior is insignificant relative to viscous behavior in synovial fluid at frequencies less than 0.1 Hz.[16] Thus, flow properties in this range may be described using viscous parameters alone.

Using a similar logarithmic scale to that used in the steady-shear measurements, ten measurements were taken per decade of frequency variation. In Hz, the frequencies employed belonged to the geometric series {0.100, 0.125, 0.158, ... 1.58, 1.99, 2.50, 3.15, 3.96, 5.00, ... 15.8, 19.9, 25.0}. Viscoelastic parameters varied slowly with frequency, so that more than ten measurements per decade would render no additional useful information to characterize the fluids. Since measurements were made at 0.5, 2.5, and 5.0 Hz, no interpolation was necessary to determine these parameters (when they were measurable). Interpolation by up to 12% of the value of the nearest measured point was required to obtain f_c and G_c (*i.e.*, if f_c = 11.2 Hz, the nearest measurements were made at 10.0 Hz and 12.5 Hz, each approximately 12% different from 11.2 Hz). Interpolation was performed using the calculations of Appendix C.

These parameters were measured using sinusoidal torque inputs throughout the range of the CSL 500, at 25, 50, 100, 200, and 300 μNm. These torques corresponded to shear stress amplitudes of 0.19, 0.39, 0.78, 1.6, and 2.4 Pa (See Appendix D for calculations). Preliminary experiments showed that, for most samples of joint fluid, it was possible to measure G' and G'' using at least one of these inputs for most of the frequencies in the range studied. When measuring viscoelastic parameters using these five inputs, five curves of storage and loss moduli were obtained. These curves would only coincide if all five inputs resulted in motion within the linear viscoelastic range, a result that did not typically occur in joint fluid experiments.

In order to verify the measurements, the output waveform was analyzed visually. When the torque input was not sufficiently large, noise overwhelmed the sinusoidal

output, rendering it impossible to decompose the signal into storage and loss components. Often the G' and G'' outputs were infinitesimal or negative values in such cases. Any data point in which the output signal was visibly different from a sinusoid, either in shape or amplitude, was discarded.

On the other hand, when the torque input is too large, motion exceeds the linear range for the fluid, and the moduli deviate from their linear counterparts.[7] This deviation cannot easily be determined by visual inspection of the output curves. It appears, however, that deviation beyond the linear range results in an underestimation of G', as determined by measurements at smaller input torques. This finding is shown below in Fig. 3.4.2.

In many cases, identical or closely approximate (within ~5%) values of G' and G'' were obtained at a single frequency with different torque inputs. These data verified the chosen torque and frequency ranges as producing strains within the linear viscoelastic range for these samples of joint fluid. In cases in which different torque inputs produced apparently sinusoidal outputs and disparate values of G' and G'' at a given frequency, the smaller torque input was assumed to represent more nearly the linear viscoelastic properties of the fluid. Preliminary experience showed that the former case occurred in almost all cases involving strain amplitude less than 0.6 (stain amplitude is a dimensionless parameter). Furthermore, the latter case occurred in almost all cases involving strain amplitude greater than 0.6. Consequently, we concluded that storage and loss moduli tend to be independent of strain amplitude at strains less than this value. This range is similar to the range employed by Safari *et al.*[17] Whenever data were available at more than one shear stress, only those resulting in strains less than 0.6 were used.

3.4.9 Conclusions Regarding Methods

Using the methods described herein, it was possible to achieve accurate estimates of steady-shear viscosity and linear viscoelastic properties of joint fluid samples, though substantial error is present. Consequently, results were accepted to two significant digits of precision, though calculations were performed using more precise figures. The most significant sources of error were due to non-homogeneity of joint fluid (Appendix E.2) and use of the rheometer below 0.5 Pa. The maximum recorded variability in ln η_0 due to non-homogeneity of joint fluid aliquots is 0.2 (ln Pa s), with underestimation more likely than overestimation. The maximum recorded error in $\dot{\gamma}$ due to use of the rheometer at low shear stress is 10%, with overestimation of η more likely than underestimation. Further discussion of potential sources of error is given in Appendices B and E.

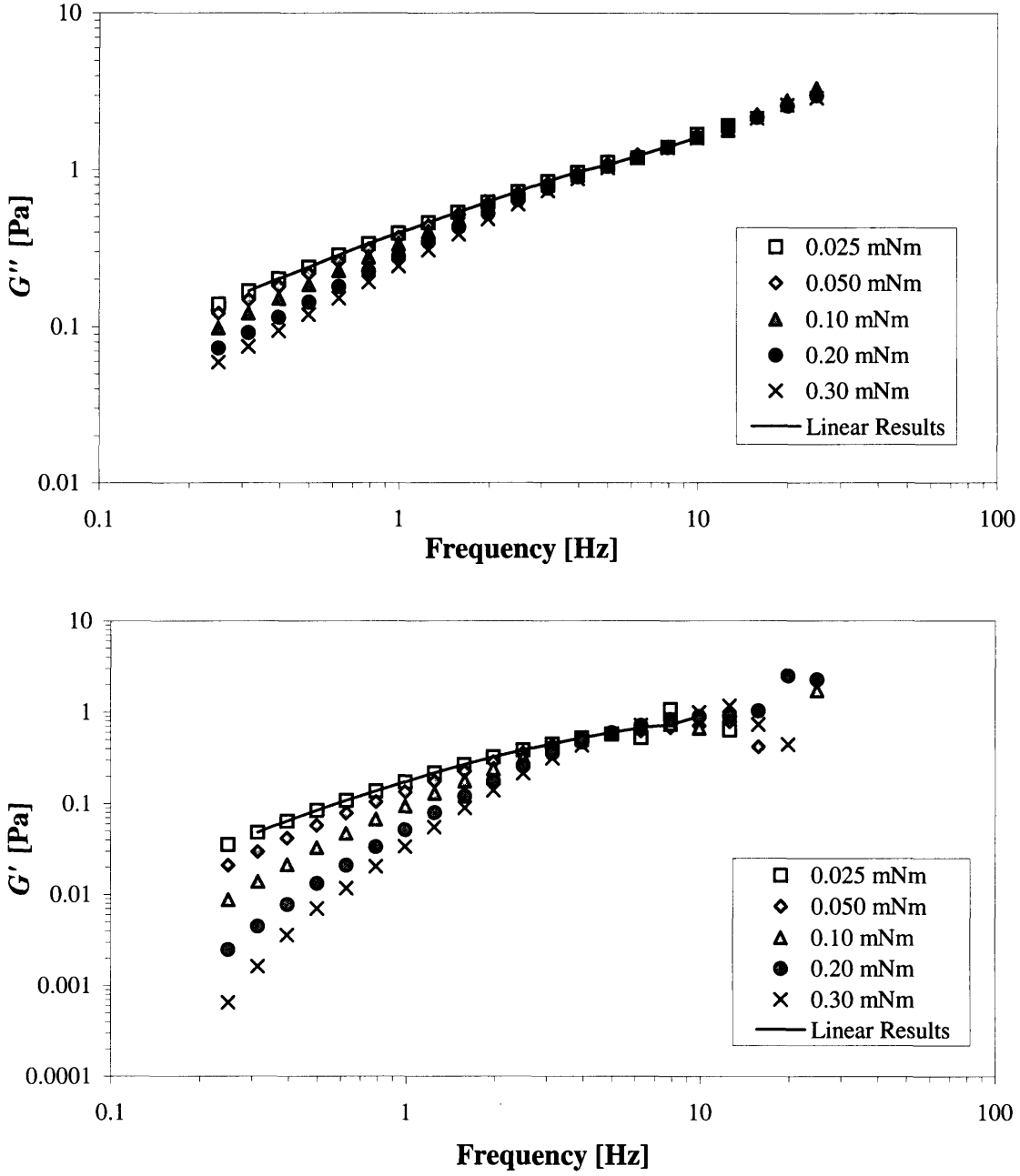

Fig 3.4.2 Loss (above) and storage (below) moduli at several shear stresses Storage and loss moduli measured at several shear stresses for synovial fluid sample obtained at index TKA from a 68 year old man with OA (Study ID H17). The linear results were compiled from the five shear stresses as discussed in the text. Throughout most of the range, the smallest input was closest to the true linear viscous response. At higher frequencies, the loss measurements converged, and all inputs gave the linear viscous response. At low frequencies, nonlinear behavior was more evident in storage modulus than in loss modulus. At the highest frequencies, the true storage output was masked by noise, especially at low shear stresses.

3.5 Results

Samples varied in quantity (0.5 ml to more than 25 ml) and appearance. A gross description of all joint fluid samples is given in Appendix F. Among samples obtained at index TKA, samples that were inflammatory in appearance (darker yellow, cloudy, and translucent[5]) tended to be greater in volume that all other types. This difference was statistically significant compared to each group except the one normal-appearing sample (Fig. 3.5.1). Among samples obtained at revision, this relationship was not observed. No correlation was found between quantity of fluid and the occasion at which it was obtained. Finally, Fisher's exact test did not suggest a relationship between gross description and occasion, except that there were no septic samples obtained at revision due to the inclusion criteria of the study.

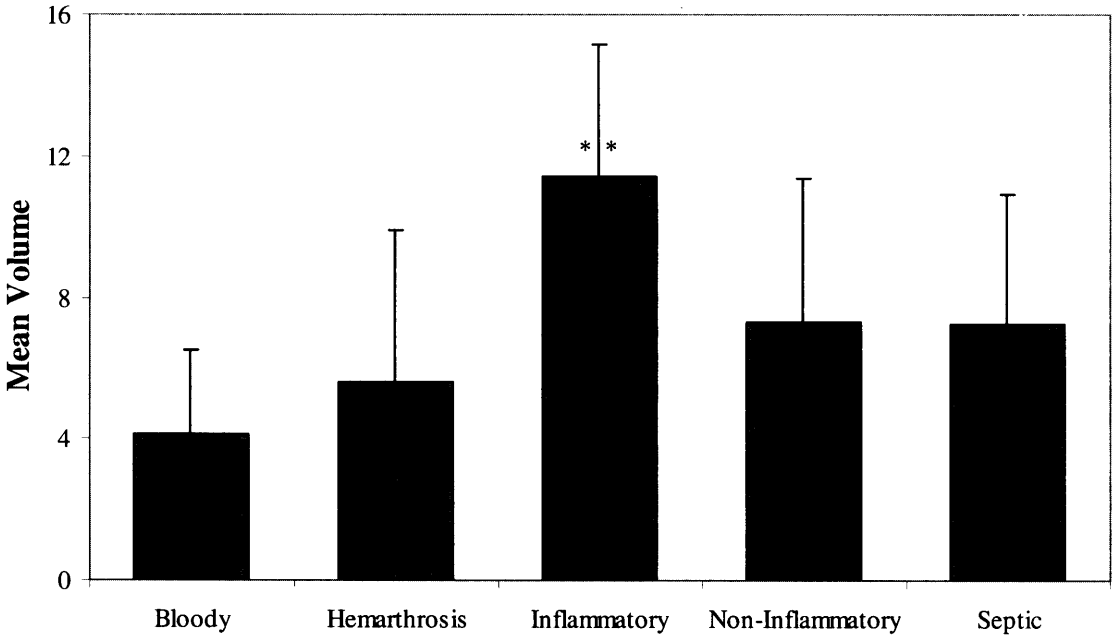

Figure 3.5.1 Mean volume and gross description of synovial fluid samples obtained at TKA Asterisks indicate that the volume difference between inflammatory fluids and each other gross category was statistically significant ($p < 0.001$). No other differences were statistically significant. Sample sizes for each group are as follows: bloody, five; hemarthrosis, nine; inflammatory, nine; non-inflammatory, 33; and septic, nine. The gross descriptions of 13 additional samples were not recorded. The lone "normal" appearing sample is not included in this figure. Error bars represent standard deviation.

3.5.1 Viscometric Parameters of Joint Fluid in the Context of TKA

The joint fluid samples generally displayed characteristic shear-thinning behavior reflected in a decrease in viscosity with increasing shear rate (Fig. 3.5.2). Although each joint fluid curve exhibited the same characteristic shape, the magnitude of the steady-shear viscosity varied over three orders of magnitude. In contrast, bovine serum exhibited only a small amount of shear-thinning throughout the test range. A typical rheogram for bovine serum is given in my master's thesis, and so not repeated here.

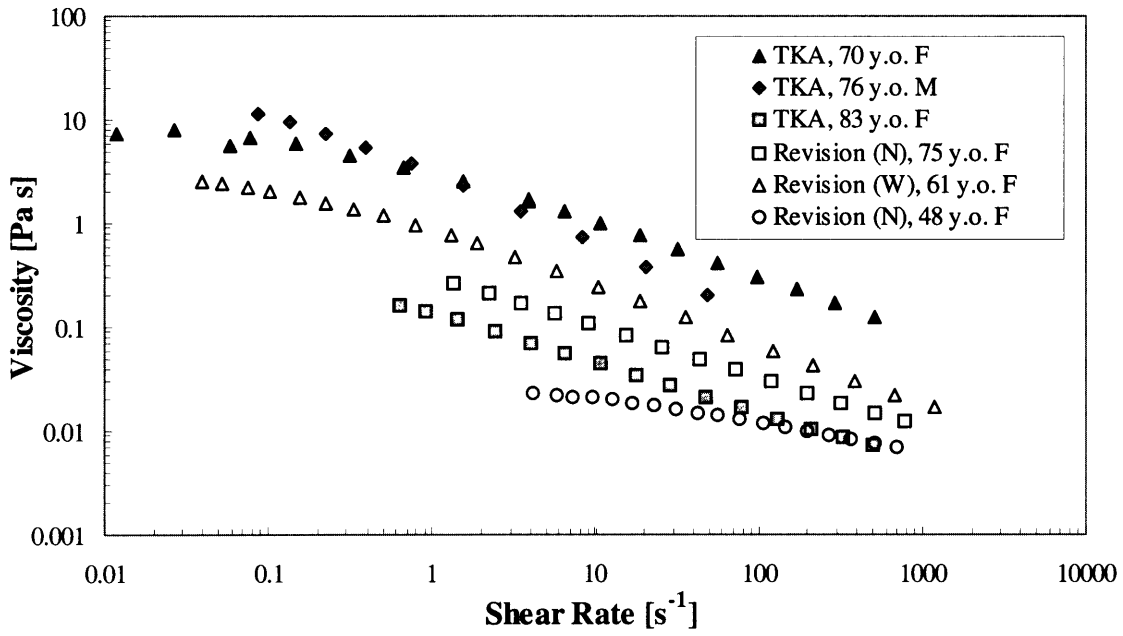

Figure 3.5.2 Steady-shear viscosity of six joint fluid samples Rheogram showing a characteristic decrease in the viscosity with increase shear rate for several samples of joint fluid from patients undergoing TKA and revision TKA. All samples exhibited shear-thinning, and all but the squares demonstrated some measure of low-shear plateau, and could be fit to the simplified Cross model. Unfortunately, it is not possible to show all viscosity-shear rate relationships on this rheogram. See Appendix G for Cross model parameters determined for each joint fluid sample. N = revision for reasons unrelated to wear. W = revision due to wear-related reasons.

In 68 cases, the joint fluid exhibited evidence of a viscosity plateau at low shear rates. In these cases, the data could be fit to the Cross model with coefficient of variation of η_0 and c less than 0.1, as calculated by chi-squared minimization. In the eight remaining cases (five index cases, two revision cases, and one case of effusion after TKA), it was not possible to obtain data at low enough shear rates to fit a low shear rate plateau. Samples that did not exhibit low-shear plateau could be fit to the Cross model, but only the rate index, d, could be determined with certainty. In each of these cases, η_0 and c had coefficient of variation greater than 1, indicating that these parameters were not well-defined by the data. As an example, Figure 3.5.3 shows one such case in which the low-shear plateau was not reached.

In addition to these cases, there were other cases in which the iterative method determined parameters, but the use of these parameters was not warranted because the data did not extend sufficiently into the low shear rate plateau. The Cross model fit was considered poor if η_0 exceeded three times the maximum viscosity measured (η_{Max}, typically at lowest shear rate). In such cases, the behavior at low shear rate did not sufficiently affect the data for the model to robustly predict the parameters η_0 and c. This criterion was met in five additional samples obtained at TKA, one additional sample obtained at revision TKA, and two samples aspirated from effused knees (one from an OA patient and one from TKA). For such samples, the value η_{Max} is shaded in Appendix G. The parameters η_0 and c were used to compare only those 60 samples that fit the Cross model. The rate index, d, was used to compare all samples. The use of the

simplified Cross model is justified both by the experience of previous work and the goodness-of-fit of most of the samples.

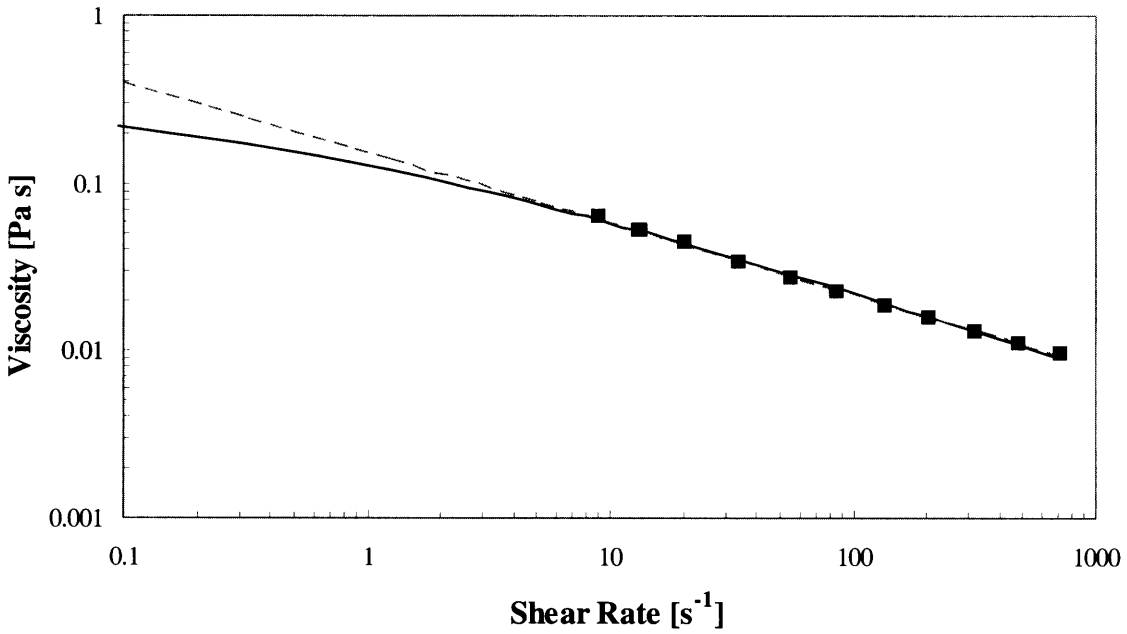

Fig. 3.5.3 Demonstration of data not exhibiting low-shear plateau In this rheogram from a 79 year old woman at TKA (Study ID 028), it is clear that the data do not approach the low shear rate plateau. Consequently, multiple sets of Cross model parameters fit the data. For example, the solid line represents $\eta_0 = 0.33$, $c = 2.3$, $d = 0.48$, and the dotted line represents $\eta_0 = 3.7$, $c = 1300$, $d = 0.44$. Consistency and η_0 could not be used for comparison among these samples. Only d and η_{1Pa} were used.

In part to compare the data in a manner that more fully includes the data that did not exhibit a zero-shear plateau, η_{1Pa} was also used to compare samples. In contrast to the other parameters, which were calculated by fitting a curve to a set of data, this parameter enabled direct data comparison. It was possible to measure η_{1Pa} for all samples. As was the case with the viscosity-stress curves, viscosity at 1 Pa shear stress (η_{1Pa}) varied over a wide range for the joint fluids. In the group of revision TKA ($n = 14$), η_{1Pa} was less than 0.8 Pa s for all samples, whereas 24% of samples obtained at index TKA ($n = 53$) had viscosity greater than 0.9 Pa s (Fig. 3.5.4). Complete results are given in Appendix G. Unfortunately, a rheogram for each joint fluid sample would take up too much space even to be included as appendices.

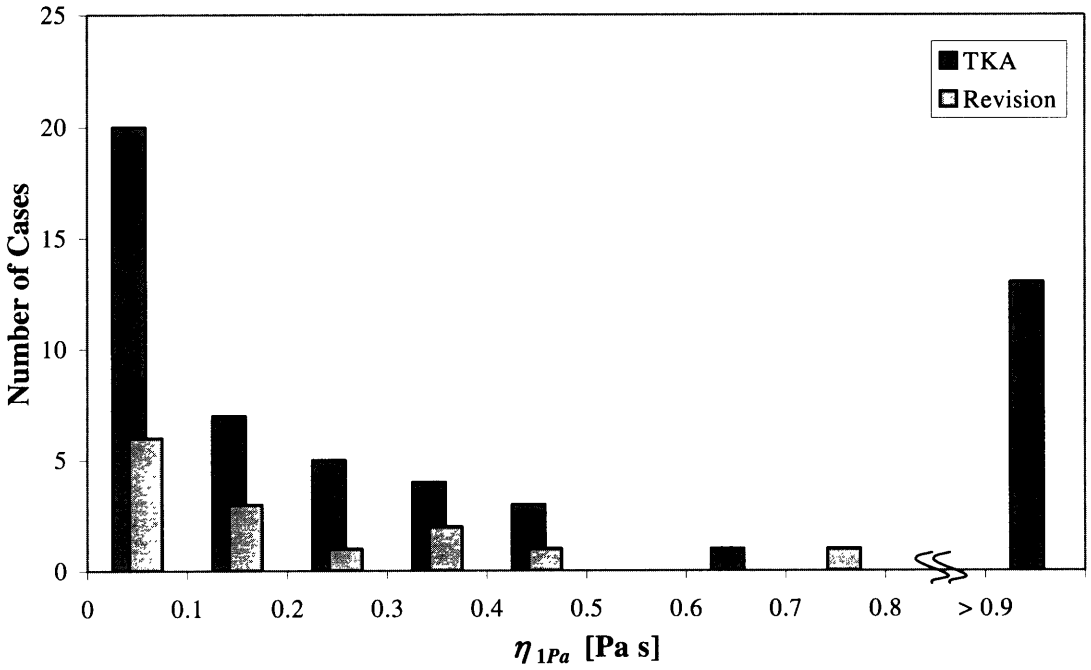

Fig. 3.5.4 Comparison of η_{1Pa} between TKA and revision TKA Histogram demonstrating the sample frequency distribution for the steady-shear viscosity at 1 Pa.

3.5.2 Four Statistical Methods for Comparing Joint Fluid Samples

Table 3.5.1 compares the parameters η_{1Pa}, η_0, c, and d for joint fluids at TKA and at revision TKA. The distributions of η_{1Pa}, η_0, and c were highly skewed toward the low end of their range, and did not form a Gaussian distribution. Consequently, mean and standard deviation are not appropriate metrics by which to compare these data. This was not the case for d. Since data regarding the properties of the two hyaluronate preparations and bovine serum were determined with little variation, it is appropriate to compare them using their mean values. These are also given in Table 3.5.1.

Joint fluid obtained at revision TKA displayed a lower viscosity compared to the index TKA samples, and began to undergo shear-thinning at a higher shear rate (Table 3.5.1). For each of the parameters η_0, η_{1Pa}, and c, differences were suggested by the data according to the Mann-Whitney test,[18] but were not demonstrated (η_0, $p = 0.057$; η_{1Pa}, $p = 0.11$; c, $p = 0.14$). By contrast, a difference *was* demonstrated between the index and revision groups by comparison of d of the groups using analysis of variance (ANOVA). The viscosity of revision TKA samples exhibited a smaller dependence on shear rate than the viscosity of index TKA samples did ($p = 0.045$). Interestingly, when only the samples fitting the Cross model were included in the analysis, the significance of the difference increased ($p = 0.023$). This reflects the fact that the most outlying samples at primary TKA (those that exhibited the least shear-thinning) most often did not fit the Cross model.

For other groups, an insufficient number of samples were evaluated to make meaningful statistical comparisons. The samples followed the same general trend as

samples from revision surgery, in that they tended to be less viscous and shear thin less than samples obtained at primary TKA.

Table 3.5.1 Flow properties of different groups of joint fluids All joint fluid data are presented as median (range) except where noted. Sample size is given as first all samples, then only those that fit Cross model, as discussed above in the text. TKA = sample obtained at index arthroplasty. Revision = sample obtained at revision of TKA. Uni-TKA = uni-compartmental TKA. [a]Values do not include samples which did not fit the Cross model; [b]Mean ± standard deviation; [c]69 year old male, hemarthrosis (Study ID 030); [d]71 year old male (Study ID 167); [e]Mean values only

	η_{1Pa} (Pa s)	η_0 (Pa s)[a]	c(s)[a]	d[b]
TKA (n = 53, 43)	0.18 (0.0094 – 17)	1.6 (0.087 – 76)	3.9 (0.047 – 91)	0.54 ± 0.09
Revision (n = 14, 11)	0.13 (0.0043 – 0.77)	0.70 (0.0087 – 4.0)	2.5 (0.0043 – 11)	0.48 ± 0.11
Effusion (n = 5, 4)	0.11 (0.039 – 0.18)	0.65 (0.43 – 0.71)	1.7 (0.85 – 4.3)	0.59 ± 0.16
Effusion after TKA (n = 3, 1)	0.010 (0.0074 – 0.18)	2.7[c]	37[c]	0.39 ± 0.10
Revision Uni-TKA[d]	0.31	1.3	2.7	0.56
Supartz[e]	3.0	3.1	0.056	0.78
Orthovisc[e]	37	39	1.0	0.71
Bovine Serum[e]	0.0015	N/A	N/A	N/A

There are at least three additional methods to meaningfully compare these groups, and these are described below. Since rheological properties are typically plotted on a double logarithmic graph, it is not unreasonable to compare the means of the natural logarithms of these data. In support of this approach, the parameters η_0 and c, both of which are converted to logarithms by the plotting method, are the ones that did not fit a Gaussian distribution. Rate index, which is made linear by the plotting method, appeared to fit a Gaussian distribution, and so was not compared in this manner.

The logarithms of these data, given below in Table 3.5.2, are compared using ANOVA. Again, cases at revision were less viscous than at index TKA, and did not shear-thin until higher shear rates; by this means of comparison, the differences in η_0 and c were statistically significant ($\ln c$, $p = 0.030$; $\ln \eta_0$, $p = 0.024$), and a difference in η_{1Pa} was suggested ($p = 0.073$). Except for $\ln \eta_{1Pa}$, these differences are statistically significant despite high standard deviations in all groups and parameters. Effusion cases had parameters similar to samples obtained at revision TKA.

Table 3.5.2 Flow properties of different groups of joint fluids, by natural log All joint fluid data are presented as mean ± standard deviation. Sample sizes are given as all, then the number fitting the Cross model. [a]Values do not include samples which did not fit the Cross model

	ln η_{1Pa} (ln Pa s)	ln η_0 (ln Pa s)[a]	ln c(ln s)[a]
TKA (n = 53, 43)	-1.6±1.8	0.38±1.53	1.3±1.4
Revision (n = 14, 11)	-2.5±1.5	-0.88±1.90	0.67±2.29
Effusion (n = 5, 4)	-2.4±0.6	-0.52±0.23	0.58±0.70
Effusion after TKA (n = 3, 1)	-3.7±1.7	1.0	3.6

Others have evaluated the viscosity of synovial fluid obtained from individuals that were categorized as: "normal," "degenerative," and "chronically inflamed."[15,19-22] In these studies, synovial fluid from asymptomatic patients consistently exhibited higher viscosity than synovial fluid from patients with degenerative or inflammatory disease. As a third means of comparison, the samples studied here were fit to these established ranges (Table 3.5.3). Since some data fit *between* the previously established ranges, the ranges were extended so that the ranges included all measured viscosities. The ranges were extended geometrically, not arithmetically, to remain consistent with the use of a logarithmic scale to compare the data.

Both groups of joint fluids were most likely to fit in the diseased range, rather than the normal or inflamed range. Using the Chi-square test, differences were seen between the two groups, with joint fluid obtained at index TKA more likely to exhibit "normal" η_0 than fluid obtained at revision TKA. This difference was statistically significant both when all data were included (p = 0.0015) and when only the data fitting the Cross model were included (p = 0.0009). The data for η_{1Pa} did not demonstrate a difference between the groups (p = 0.15).

Table 3.5.3 Flow properties of different groups of joint fluids fit to historical controls Each group is given as the percentage of samples that fit within the range of the prescribed historical controls. In the η_0 comparison, the first percentage and sample size include all samples, and the second percentage and sample size include only those samples which fit the Cross model.

		Range	*TKA* (n = 53, 43)	*Revision* (n = 14, 11)
η_0	Normal	> 2.5 Pa s	36%, 37%	14%, 9%
	Degenerative	0.1 to 2.3 Pa s	64%, 63%	64%, 63%
	Inflammatory	< 0.1 Pa s	0%, 0%	21%, 27%
η_{1Pa}	Normal	> 2.3 Pa s	11%	0%
	Degenerative	0.03 to 1.7 Pa s	77%	71%
	Inflammatory	< 0.03 Pa s	11%	29%

Finally, in order to characterize joint fluid in TKA, it is reasonable to consider the 5th percentile and 95th percentile for each parameter. In a normally distributed group, this would correspond to between one and two standard deviations above and below the mean value. In these groups, it indicates that 9 out of 10 joint fluid samples from TKA or from revision TKA fit into this range. Table 3.5.4 summarizes the range of values determined

for each group from these experiments. Only TKA and revision TKA are considered in this comparison because the other groups were not sufficiently large for meaningful analysis.

Table 3.5.4 Flow properties of different groups of joint fluids, 5th to 95th percentile All joint fluid data ranges, from 5th percentile to 95th percentile, are presented below. Values in parentheses only include samples which fit the Cross model.

	η_{1Pa} (Pa s)	η_0 (Pa s)[a]	c (s)	d
TKA, $n = 53$ ($n = 43$)	0.022 – 3.2	0.17 – 25.4 (0.17 – 9.0)	0.46 – 340 (0.46 – 19)	0.40 – 0.67
Revision, $n = 14$ ($n = 11$)	0.013 – 0.42	0.043 – 4.0 (043 – 1.6)	0.13 – 94 (0.13 – 6.2)	0.36 – 0.59

3.5.3 Correlations among Flow Properties

Using regression analysis, each viscous parameter was strongly correlated to the others when only samples that fit the Cross model were considered ($p < 0.0001$). In all cases, the relationship between the parameters was weakened by including data which did not fit the model. Perhaps trivially, η_{1Pa} and η_0 were positively correlated for all samples ($R^2 = 0.52$ for a power law fit); when only those fitting the Cross model were included, the measures correlated by the power 1.00 ($R^2 = 0.89$). This relationship is given graphically below in Fig 3.5.5.

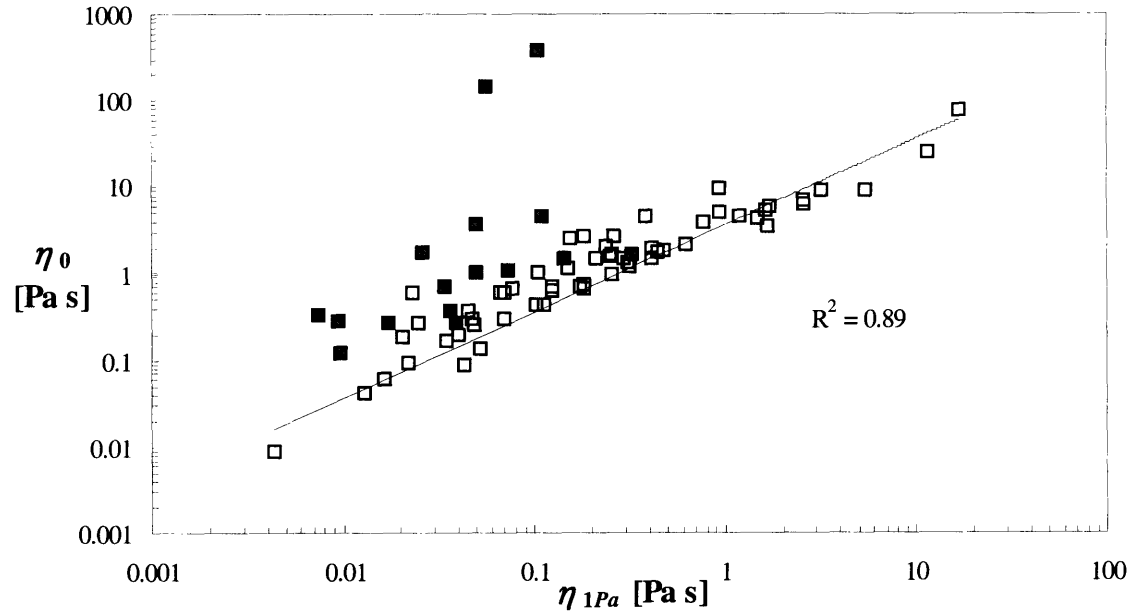

Fig. 3.5.5 Correlation between η_0 and η_{1Pa} Double logarithmic graph showing the relationship between two measures of viscosity. Open squares represent those data which fit the Cross model. Closed squares represent those data which did not fit the Cross model. The curve shown is a linear fit through the data. The discussion in the text describes a power law fit, but the power law relationship is equivalent to the linear fit shown here.

Due to the strong correlation between η_0 and η_{1Pa}, only comparisons between η_0 and other parameters are discussed; in each case, the relationship between parameters would be unchanged by the use of η_{1Pa} instead of η_0. The one exception is that d correlated with η_{1Pa} for all samples (exponential regression, R^2 = 0.57) better than it correlated to η_0. Low shear rate viscosity correlated to d by a power law relationship for all samples (R^2 = 0.33 for all data, 0.77 for data which fit the Cross model well; Fig. 3.5.6).

Of the parameters, c correlated the most poorly with the others. For example c positively correlated to η_0 by a power law relationship, with (R^2 = 0.51 for all data, 0.70 for data which fit the Cross model well). Consistency and d correlated only when considering those data which fit the Cross model (R^2 = 0.38). Figures 3.5.5 (above) and 3.5.6 (below) demonstrate typical graphs of correlations between parameters.

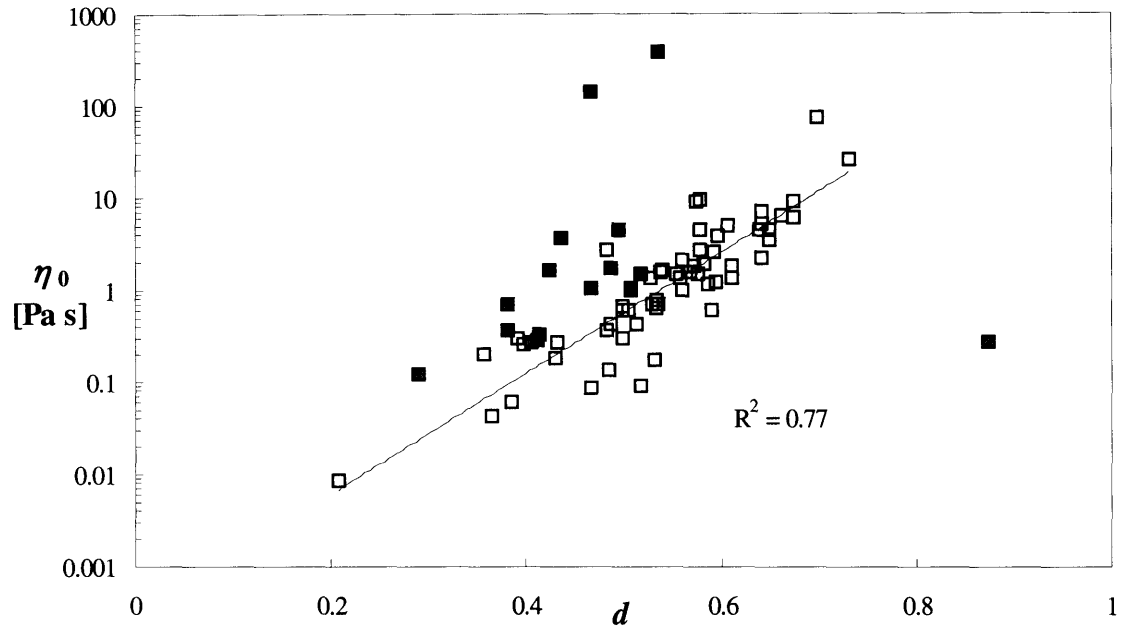

Fig. 3.5.6 Correlation between η_0 and d Logarithmic graph showing the relationship between viscosity at low shear rate and rate index. Open squares represent those data which fit the Cross model. Closed squares represent those data which did not fit the Cross model. The curve shown is an exponential fit through the data which fit the Cross model, as discussed in the text.

No correlation could be found between any viscous parameter and age, gender, or involved leg. Moreover, there was no correlation between the viscous parameters and the volume of joint fluid. There was no correlation between gross description and any viscous parameter. Stratifying the joint fluid samples obtained at revision TKA into wear-related revision (n = 8) and revision for reasons other than wear (n = 6) revealed no difference between the groups in any of the measured or calculated parameters.

3.5.4 Bilateral TKA

In six cases, steady shear viscosity was measured in synovial fluid obtained from both knees of one patient. In five of these cases, synovial fluid was obtained from both knees during bilateral TKA. In the other case, synovial fluid was obtained from one

knee, then the other during subsequent TKA. The flow properties of each of these samples are given below in Table 3.5.5. In each case, the properties of joint fluid varied substantially from knee to knee, the most similar case being Study ID 143 and 145, in which fluid from the right knee was approximately twice as viscous as that from the left. Since only one case was examined in which fluid was obtained with the progression of time, no conclusions can be drawn as to changes in synovial fluid with time.

Table 3.5.5 Properties of multiple joint fluid samples obtained from the same patient In all but the last case, samples were obtained from both knees during bilateral TKA. In the last case, samples were obtained during successive unilateral TKAs. The numbers underneath the patient demographic represent study ID numbers for reference with the appendices.

Patient	*Time Span*	*Leg*	η_{1Pa} *(Pa s)*	η_0 *(Pa s)*	*c* (s)	*d*
52 year old ♀ (152 & 153)	Simultaneous	Right	0.44	1.8	2.8	0.61
		Left	1.7	6.0	7.2	0.67
68 year old ♀ (155 & 156)	Simultaneous	Right	0.052	0.13	0.13	0.48
		Left	0.41	1.9	3.9	0.58
68 year old ♂ (H07 & H08)	Simultaneous	Right	0.056	N/A	N/A	0.47
		Left	0.104	1.0	6.2	0.51
73 year old ♀ (148 &146)	Simultaneous	Right	0.073	N/A	N/A	0.47
		Left	0.32	N/A	N/A	0.42
89 year old ♀ (143 & 145)	Simultaneous	Right	0.62	2.2	2.8	0.64
		Left	0.31	1.4	2.3	0.61
45 year old ♀ (H03 & H09)	4 months	Right	0.24	2.1	9.6	0.56
		Left	0.92	5.0	11	0.61

3.5.5 Thixotropy

Although measuring thixotropy was not a primary goal of this thesis, thixotropic behavior was measured in two joint fluid samples using the protocol of Oates *et al.*[14] The first case is shown below in Fig. 3.5.7. The other case is discussed in Appendix E, and was used as an example of the repeatability of measurement. In the first case, no low shear rate plateau was determined using the standard protocol, but the Oates protocol generated a limiting apparent viscosity. In the second case, a low shear rate plateau was observed using both protocols. In both cases, the behavior at high shear rate was identical using both protocols.

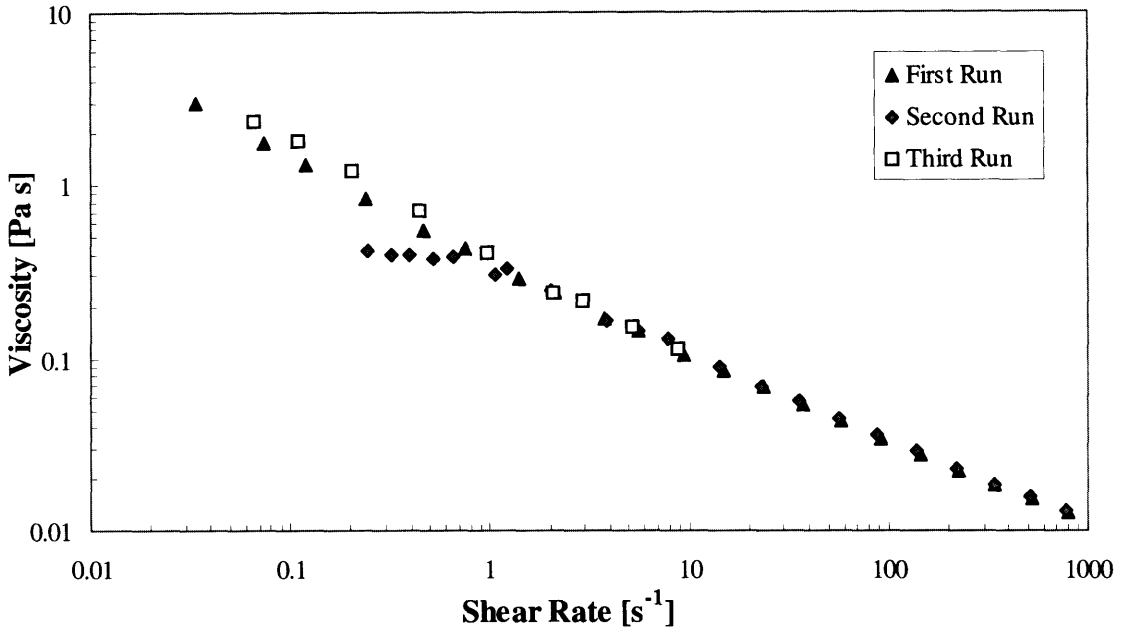

Fig. 3.5.7 Thixotropy in joint fluid Three successive measurements of viscosity using different protocols demonstrate thixotropy in this sample of joint fluid from a 60 year old man at index TKA (Study ID H19). First, steady shear viscosity was measured using the standard protocol discussed in section 3.4.1 (solid triangles). Second, apparent viscosity was measured in 80-second averages over an increasing sweep from 0.1 to 10 Pa of shear stress (shaded diamonds). Finally, steady shear viscosity was measured again over a decreasing sweep from 1 to 0.1 Pa of shear stress (empty boxes).

3.5.6 Temperature

In certain simple solutions, time and temperature can be superimposed on a rheogram. Specifically, decreasing the measurement temperature can shift the low shear rate plateau so that it occurs at higher shear rates. For those cases in which a low shear rate plateau could not be observed, it was reasonable to make measurements at a lower temperature in an effort to shift the low shear rate plateau into the region of shear rates evaluated. Tests were conducted at 15°C on a synovial fluid sample obtained at TKA from a 66 year old woman (Study ID H23) and at 20°C on a synovial fluid sample obtained at TKA from a 68 year old man (Study ID H17). The results from these tests are summarized below in Table 3.5.6. Reducing the temperature increased the viscosity at all shear rates (an upward shift), and had little effect on the rate of shear-thinning. Measuring viscosity at this reduced temperature did not improve the robustness of the Cross model, however. That is, this method did not reduce the gap between η_{Max} and η_0, one measure of to what extent the experiment approaches the low shear rate region. Since making measurements at a lower temperature did not improve the robustness of the Cross model, all other experiments were performed at 25°C to remain consistent with what others had done and close to clinically relevant temperatures.

Table 3.5.6 Properties of two joint fluid samples measured at different temperatures In both cases, viscosity was higher at all shear rates when temperature was reduced. Rate index was not greatly affected by the temperature change, and the effect on *c* was not clear. The fit to the Cross model was not improved using this method, though both samples fit the Cross model at the higher temperature. The numbers underneath the patient demographic represent study identification numbers for reference with the appendices.

Patient	*Temperature*	η_{1Pa} *(Pa s)*	η_0 *(Pa s)*	*c* (s)	*d*	η_{Max} *(Pa s)*
68 year old ♂ (H17)	25°C	0.049	0.26	1.9	0.40	0.12
	20°C	0.061	0.30	1.6	0.41	0.15
66 year old ♀ (H23)	25°C	0.047	0.30	3.6	0.39	0.13
	15°C	0.074	0.56	7.4	0.41	0.22

3.5.7 Viscoelastic Parameters

The linear viscoelastic curves for joint fluid samples displayed a characteristic shape (Fig. 3.5.8). At low frequencies, the loss modulus dominated over the storage modulus. As the imposed frequency was increased, the storage modulus and loss modulus both increased. Table 3.5.7 summarizes the storage and loss moduli of joint fluid samples from all groups tested at three physiologically relevant frequencies: 0.5, 2.5, and 5.0 Hz. Complete results are given in Appendix H.

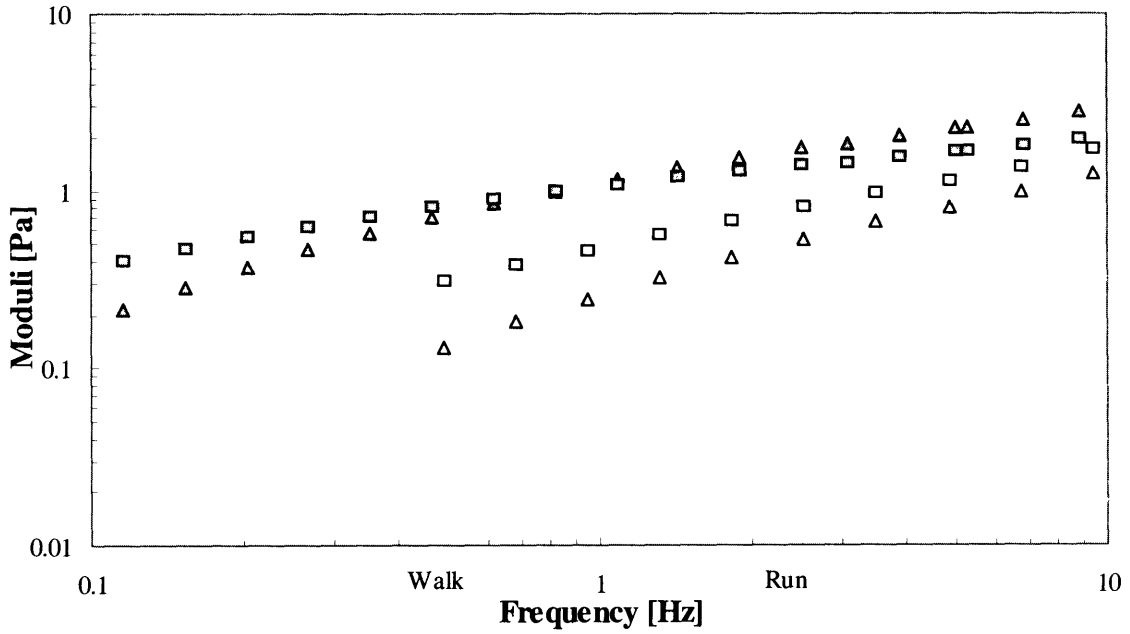

Fig. 3.5.8 Typical curves representing the change in storage modulus (triangles) and loss modulus (squares) with frequency of oscillation Solid shapes represent a sample obtained from an 89 year old woman at index TKA (Study ID 145). This sample exhibited viscoelastic crossover at 0.87 Hz. Hollow shapes represent a sample obtained from a 72 year old man undergoing revision TKA for wear-related osteolysis (Study ID 019). The second sample did not exhibit viscoelastic crossover within the range tested. Crossover in the first sample occurred within the range of frequencies encountered *in vivo* (given by dashed lines).

In many cases, the storage modulus was too small to be measured at low frequencies. In these cases, it is assumed that the moduli at low frequencies are less than or equal to those measured at higher frequencies. Due to the uncertain parameters in these cases, a report of the mean value of these data (exclusive of those unknown results) would be skewed toward the high end. Consequently median and 5^{th} – 95^{th} percentiles are used. This analysis shields the results from the effects of outlying data points.

At low frequencies, loss modulus exceeded storage modulus. As frequency was increased, both moduli increased, but storage modulus typically increased more rapidly, such that at high frequencies ($f > f_c$), the storage modulus dominated the response in many samples. The crossover frequency (f_c), at which the storage and loss moduli are equal, has been used to characterize the relative importance of elastic and viscous effects in fluids for which crossover existed. This frequency corresponds to the frequency at which the phase angle δ between the imposed stress and resulting strain is 45° (*i.e.*, $\tan \delta = G'/G'' = 1$). It was possible to measure the crossover in 23 of 36 joint fluid samples obtained at TKA and seven of eleven joint fluid samples obtained at revision (Table 3.5.7). In the other seventeen samples obtained on these occasions, the storage modulus was not sufficiently large, even at high frequency, to measure a crossover. In this table, the viscoelastic properties of Supartz are included for comparison. Although not reported in Table 3.5.7, viscoelastic properties were measured in samples obtained on other occasions as well. These data are presented in Appendix H, and are used for correlation with steady-shear viscosity parameters.

Table 3.5.7 Viscoelastic properties for joint fluid samples Original data are presented as median (5^{th} – 95^{th} percentile), except joint supplements, which are presented as means only. Modulus at crossover is only compared among samples that exhibited crossover, and is presented as mean ± standard deviation. All moduli are presented in Pa. "Crossover" = fraction exhibiting crossover. y.o. = year old. NR = Not reported. "52-78 y.o." are a historical healthy control group,[16] presented as mean value only. [a]Results were given in dynes/sec^{-1}, which is assumed to be an editing error for Hz, based on an accompanying graph. [b]Results were given in "dynes/sec^{-2}," which is assumed to be an editing error for dynes/cm^2 (0.1 Pa), based on an accompanying graph.

Group	*TKA*	*Revision*	*Supartz*	*52-78 y.o.*
Crossover?	23/32	7/11	Yes	Yes
f_c (Hz)	14 (1.5 – None)	25 (11 – None)	11	0.4[a]
G_c	1.3 ± 0.6	1.3 ± 0.5	30	6[b]
$G'_{0.5Hz}$	0.46 (0 – 2.4)	0.34 (0 – 0.57)	1.8	NR
$G''_{0.5Hz}$	0.56 (0 – 2.1)	0.45 (0 – 0.70)	6.5	NR
$G'_{2.5Hz}$	1.1 (0.21 – 5.0)	0.98 (0.073 – 1.5)	12	20[b]
$G''_{2.5Hz}$	1.1 (0.34 – 3.2)	1.1 (0.23 – 1.8)	20	10[b]
G'_{5Hz}	1.6 (0.38 – 6.4)	1.4 (0.074 – 2.2)	21	NR
G''_{5Hz}	1.4 (0.49 – 3.7)	1.4 (0.40 – 2.3)	22	NR

The data suggested a difference in crossover frequency between samples obtained at index TKA and samples obtained at revision TKA. However, the difference was not statistically significant (Student's *t*-test, $p = 0.053$). Previously it was found that normal joint fluid for patients in the age group likely to have TKA crossed over from viscous to

elastic at frequencies an order of magnitude lower than either group presently studied.[16] No difference was demonstrated between the two groups with regard to modulus at crossover or either storage or loss modulus at any of the frequencies studied, either by Student's *t*-test or by the Mann-Whitney test, though a difference was suggested by $G'_{0.5Hz}$ ($p = 0.088$). Finally, samples at TKA were no more likely to exhibit crossover within the range studied than samples at revision. Importantly, compared to reports in healthy patients, viscoelastic properties were markedly degenerate in the patient populations presently studied.

3.5.8 Correlation between Viscous and Viscoelastic Parameters

In 45 samples, viscous and viscoelastic parameters were both measured. Several viscoelastic parameters were highly correlated with viscous parameters, especially when only samples fitting the Cross model were included. For example, when the six cases not fitting the Cross model were excluded, both storage and loss moduli at 0.5, 2.5, and 5 Hz were highly correlated to η_0, η_{1Pa}, c, and d ($p < 0.0001$), though the correlations between parameters were typically not well described by a linear relationship. When these six cases were included, the correlation between modulus and c was no longer significant, but correlations remained among the other parameters. As an example, the relationship between $G'_{0.5Hz}$ and η_{1Pa} is given below in Fig. 3.5.9. The 23 other correlations between viscous and viscoelastic parameters are not shown, as they would merely fill reams of paper with similar graphs.

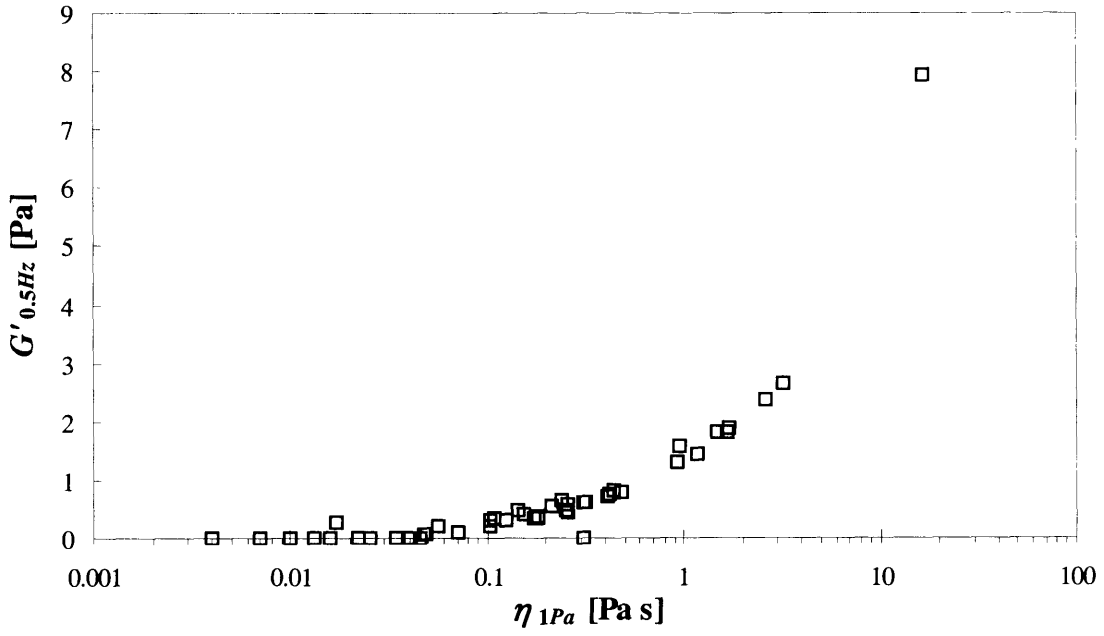

Fig. 3.5.9 Correlation between $G'_{0.5Hz}$ and η_{1Pa} Logarithmic graph showing the relationship between elastic modulus at low frequency and viscosity at 1 Pa shear stress. For viscosity less than 5 Pa s, no storage modulus could be measured. Above this value, elasticity increased substantially. The relationship appears more dramatic by the use of a logarithmic scale. Similar results were found in relating other viscous and viscoelastic parameters.

Using Fisher's exact test, viscosity range correlated with the existence of a viscoelastic crossover within the measured range, with more viscous samples being more likely to exhibit crossover (Table 3.5.8). Among those samples for which crossover between G' and G'' could be measured, crossover occurred within the range of frequencies encountered physiologically. Although crossover did not occur within this range for all samples, both storage and loss moduli were of the same order of magnitude throughout this range in many samples.

Table 3.5.8 Comparison of viscoelastic crossover versus viscosity range Fluids with "normal" viscosity almost always exhibited viscoelastic crossover, whereas fluids with very low, or "inflammatory" viscosity never exhibited sufficient energy storage to lead to crossover.

Crossover	*Normal*	*Degenerative*	*Inflamed*	*p*-value
η_0	11/12	19/30	0/3	0.0085
η_0 (cross only)	10/10	18/26	0/3	0.0030
η_{1Pa}	3/3	27/34	0/8	< 0.0001

3.5.9 Use of Viscoelastic Parameters to Estimate η_0

For samples that failed to fit the Cross model, it may have been possible to estimate η_0 from viscoelastic parameters. Specifically, using loss modulus at low frequency, $\eta_0 = \lim_{f \to 0} G''/2\pi f$. From a cursory examination of two cases which did not fit the Cross model well (Study ID 170 and 171), it is clear that the frequencies at which G'' is measured are not sufficiently low to make a useful estimate. Therefore, such an analysis was not performed.

3.6 Discussion

Clear answers were determined to the two major questions of this study. First, viscous and viscoelastic properties varied widely in the patient populations studied. Second, differences were demonstrated between the groups of fluids obtained at TKA and those obtained at revision TKA, though the differences between these groups were small relative to the variability within each group.

3.6.1 Variability of Viscous and Viscoelastic Parameters of Joint Fluid

The viscous properties of synovial fluid obtained at index TKA and revision TKA varied widely, and were degenerated with respect to synovial fluid from healthy patients as previously published. A comprehensive study of viscoelastic properties of normal and diseased synovial fluid had not previously been conducted. However, viscous and elastic moduli at 2.5 Hz as well as crossover frequency and modulus in normal synovial fluid have been reported.[16] Compared to normal, all modulus parameters were markedly decreased in patients undergoing index and revision TKA. Moreover, crossover frequency was increased in these arthroplasty fluids compared to normal, indicating that viscosity is more likely to dominate over elasticity at frequencies encountered in replacement joint articulations.

The viscous parameters of synovial fluid taken at revision TKA spanned a wide range, η_0 covering almost three orders of magnitude. The variability is demonstrated by

the large range of viscous parameters, the relatively high standard deviations of these parameters, even when evaluating the logarithms of the data. The 5^{th} – 95^{th} percentile ranges are an excellent statistical tool to describe the true variability of the groups, because they show over what range most samples fall without being too overwhelmed by outliers.

The demonstration of flow property variability, coupled with unexplained variation in prosthetic wear rates observed *in vivo*,[23] raises the question of the tribological importance of joint fluid flow properties, both in shock absorption and in fluid film lubrication. In particular, a connection between viscosity and wear is supported. This issue warrants the additional study of wear test lubricants with different rheological properties to determine the effect of the shear viscosity on wear rates in TJA.

Notably, in 26 of 79 patients undergoing TKA and six of twenty cases at revision, less than 2.5 ml of joint fluid could be removed for evaluation. In addition to these, there were numerous cases in which there was not a sufficient amount of fluid for the surgeon to obtain. This raises the question of the effect of joint fluid volume in the wear of total knee replacement prostheses. Even though all fluid present in the knee could not be removed, the volumes recorded represent a reasonable estimate of the amount of fluid present in the joint. Furthermore, the quantities obtained are consistent with the observations of others regarding the quantity of synovial fluid in symptomatic and asymptomatic joints.[24] No work has been conducted to correlate fluid volume to the tribology of TKA, though a strong connection is recognized in other (non-biological) articulations. One might expect, for example, that regardless of the fluid properties, its presence would reduce adhesive wear and aid in the removal of wear particles, thus reducing third-body wear.

Interestingly, for several patients, the viscosity of synovial fluid from the right knee differed substantially from that of fluid from left knee. This result suggests that local alterations, rather than a systemic disorder, control the properties of the joint fluid in these cases. A possible source of local control of joint fluid properties is the synovial membrane, whose role in TKA has not been fully examined. This finding is also consistent with the radiological finding that, in bilateral THA, wear rate on one side predicted only 61% of wear in the contralateral side (*i.e.*, $R^2 = 0.61$).[25] Although the authors of this study concluded that patient factors could not account for 39% of the variability, the present result underscores potential differences between legs, and makes the report less meaningful. There are, of course, other factors that may distinguish the sides, including joint geometry and right- or left-sided dominance.

3.6.2 Comparison of Viscous and Viscoelastic Parameters of Joint Fluid

The hypothesis that viscous properties of joint fluid at revision TKA would be altered with respect to properties of fluid obtained at index TKA was confirmed. Viscosity, when compared to expected ranges, showed fluid from revision TKA to be degenerate with respect to fluid obtained at index TKA. Direct comparison of d and of the natural logarithm of other viscous parameters (*i.e.*, η_0, η_{1Pa}, and c) showed a clear difference between the groups despite the overwhelmingly wide range of the data. Also, by comparing the groups to historical controls, a trend toward diminished values at revision emerged from the data. Thus, the first three statistical methods used (Mann-

Whitney, Student's *t*-test on logarithms, and Fisher's exact test using historical controls as ranges) all aid in distinguishing among these two groups. A combination of analytical tools was necessary because of the highly variable nature of the data.

The viscoelastic moduli did not demonstrate differences between the two groups. Modulus at crossover was actually slightly higher at revision, but this parameter was skewed by the higher frequency of crossover in the group. Loss modulus was not different between the two groups at any frequency in the range studied. Storage modulus was somewhat lower at 0.5 Hz, but not enough to be statistically significant. The statistical techniques used to consider steady-shear viscosity would each have merit in viscoelasticity analysis as well. Such a detailed analysis was not performed because it was not necessary. Differences between the groups had been previously shown in the viscous properties.

The differences between viscous parameters at index versus revision TKA further suggested that the joint fluid in TKA patients was different from the synovial fluid present before TKA. This finding supports the work in a rabbit model that hyaluronic acid concentration did not return to normal values after arthroplasty.[26] These results warrant an examination of the composition of joint fluid after TKA to determine what brings about the differences in parameters.

The trend toward decreased viscous properties has two likely interpretations. The first and more obvious explanation is that the properties after arthroplasty differ from those before arthroplasty. This could be caused by incomplete regeneration of the synovial membrane, modified biomechanical environment, and/or the removal of soft tissue contribution to joint fluid makeup, among other things. In this case, one would expect that the fluid differs within a single patient before and after arthroplasty. Alternatively, it may be that the joint fluid is not greatly affected by arthroplasty, and it is simply that those patients with more degenerate fluid are more likely to require revision. Thus, the study may have an inherent ascertainment bias. Although this would represent a limitation of the study, as discussed below in section 3.6.6, it would support the claim that joint fluid affects TJA outcomes.

3.6.3 Anomalous and Thixotropic Samples

There are two reasons samples may not have fit the Cross model. First, there may have been a component in some joint fluid samples that interacted with other molecules, causing an almost indefinite increase of apparent viscosity. Such a relationship has been shown between HA and aggrecan,[27] which could be released from damaged cartilage into the joint space. Since joint fluid is filtered from plasma through the synovial membrane, which may variably function or dysfunction in arthroplasty patients, it is quite possible that large aggregating proteins or even clotting proteins could be allowed into joint fluid in some cases. It is interesting to note that there was no correlation between gross description and likelihood of fitting the Cross model, however, since one might expect such agglomerations to be visible grossly.

In this type of case, one would expect a viscosity-shear rate relationship such as in Fig 3.5.7 above, in which a low shear rate limit in apparent viscosity can be found, but equilibrium can not be reached. Clearly this does not occur in all joint fluid samples,

however, as shown in Appendix E.1, in which the Oates protocol[14] is used to demonstrate repeatability of measurement of η_0.

An alternative explanation for the inability to fit some samples to the Cross model relates to the development of experimental techniques. Because of the small amount of fluid obtained in many cases, it was not possible to work out the protocol without using the entirety of fluid samples obtained. As a consequence, some samples were tested under protocols less likely to achieve equilibrium, and thus less likely to produce sufficient data to demonstrate low shear rate plateau. This is evidenced in the larger frequency of results failing to fit the Cross model among the first samples tested. As the technique improved, samples were less likely to fail to fit the Cross model. This explanation does not completely explain the findings, however, since some samples that did not fit the Cross model were tested after the protocol was well defined (*e.g.*, Fig. 3.5.7).

The approach of altering temperature to achieve η_0 by time-temperature superposition was not helpful. Within the 10°C reduction measured, there was no change in the ratio of η_0 to η_{Max}. This ratio would be reduced if the use of a lower temperature improved the robustness of the model. Further reducing the temperature may have had some effect, but since the rheological properties depend on interactions among molecules, and those interactions may change in a complex fashion as temperature changes, there was some concern that measurements at different temperature would be as likely to introduce additional error as it would to provide additional information.

In summary, there is a real thixotropic effect in some joint fluid samples. This effect likely depends on particular components not typically present in joint fluid and not immediately obvious upon inspection. The present data contain some such samples, but some of the first samples tested did not fit the Cross model for reasons related to the test protocol. The present studies do not identify which molecules are most likely candidates for this interaction, but others whose work has been referenced above are currently working on this topic. What effect these molecules have on the tribology of TKA is not clear.

3.6.4 Correlations among Parameters

All viscous and viscoelastic parameters were strongly correlated. At some point, quantitative relationships between the parameters could be useful, as could a comparison between these data and correlations determined in other solutions, such as HA. At this time, however, the complexity of joint fluid and the unclear relationship between rheology and tribology makes such an analysis cumbersome and not terribly useful. From the standpoint of assaying fluid, however, it seems that any one parameter does well to estimate many other parameters. Since viscoelastic parameters tended to distinguish among lubricants less than viscous parameters, and since shear thinning appeared to be the easiest thing to measure, I would recommend a viscosity assay in which the shear thinning region of joint fluid is measured. This would enable a power law type model, with a parameter analogous to d and a parameter analogous to η_{1Pa}. These parameters could be used to characterize joint fluid, with other parameters determined from these.

Such an analysis might not show thixotropic effects. I would expect that the importance of thixotropy, if any, would be to indicate the presence of particular molecules that imply synovial membrane dysfunction. These molecules would be more reliably measured using a biochemical assay than a rheological one, since rheology is affected by so many other components.

Finally, one might expect that, recommending the use of a two parameter power law model, I should introduce quantitative relationships for all parameters. I have not done so, and will put off such a quantitative analysis until it is shown that a rheological assay for joint fluid has clinical utility. The data included in Appendices G and H enable curves such as 3.5.6 and 3.5.9 to be drawn for all viscous and viscoelastic parameters described. From these curves, quantitative relationships could be determined if necessary.

3.6.5 Bovine Serum and Hyaluronic Acid Preparations

All prosthetic joint fluids were at least one order of magnitude more viscous than bovine serum, the lubricant currently used in most laboratory wear tests. If viscosity affects wear at the shear rates encountered in the replacement joint, then bovine serum cannot mimic the *in vivo* environment in lubricating metal on polyethylene articulation. This would suggest that a lubricant should be used that has all relevant tribological properties and components in common with joint fluid. This finding warrants further study into the relative importance of fluid film lubrication on tribology of these components, and specifically the effect of viscosity and viscoelasticity on wear. In order to truly represent joint fluid, however, it is necessary for a test fluid to mimic the properties of joint fluid throughout the range of parameters relevant to TJA, including boundary lubricating properties and small gap rheological properties.

The hyaluronate preparations were more viscous than the joint fluid samples. Orthovisc was ten times more viscous than Supartz primarily due to its higher molecular weight and concentration. That the consistency of Orthovisc and Supartz was less than that of normal joint fluid samples correlates well with their molecular weights, which are smaller than that of the hyaluronic acid in normal synovial fluid. These findings were consistent with the rheological properties of hyaluronic acid, as measured by others.[28] Since the joint fluid supplements tended to be more viscous than the joint fluid samples, the addition of hyaluronic acid to a protein-containing solution could provide a mixture whose bulk flow properties more closely mimic the *in vivo* environment over the range of frequencies and deformation rates measured. Since endogenous HA contributes heavily to the viscosity of joint fluid,[29] a lubricant including HA may be a more appropriate mixture for use in wear tests.

3.6.6 Limitations of the Current Study

One limitation of this study was that flow properties were not measured under all the conditions relevant to replacement joints (*viz.*, gap between the surfaces and shear rate). The minimum gap between the cartilage surfaces in the loaded knee joint has been estimated at 0.1 μm in the natural knee,[30] much smaller than the 300 μm gap employed by the CSL 500 rheometer. It has been shown that the flow properties of fluid films on the order of hundreds of nanometers in thickness can differ from those of the bulk fluid.[12]

Furthermore, the estimated maximum shear rates in the natural knee are at least an order of magnitude higher than the range in which we have measured.[4] The shear rate dependence of the viscosity of joint fluid has been demonstrated in this work. Since it is likely that the maximum shear rate and minimum gap present in the replacement knee are different from the conditions extant during analysis, the properties measured do not completely describe the relevant behavior of joint fluid. Furthermore, extensional viscosity and normal stress differences may also be relevant to the protection of TJA, and thus would be useful to measure. Nonetheless, making an initial excursion into a new field of measurement, it was appropriate to measure selected properties in a range that enabled comparison with previous work.

Ultimately, it may be beneficial to measure the flow properties of joint fluid at very small gaps, as well as normal stress differences and extensional properties. Unfortunately, the apparatus approved for biological fluids is not capable of employing the necessary geometry for such measurements, so future experiments of such properties will likely be geared toward evaluating the properties of individual components of joint fluid or synthesized joint fluid, rather than joint fluid *per se*.

The second limitation of this study was that multiple samples were rarely obtained from the same joint of the same patient at different times. As a consequence, it is difficult to draw conclusions about changes in joint fluid over time from this study. A useful follow-up study would obtain samples regularly from a set of arthroplasty patients. These samples can be compared to show, for example, whether the high variability reflects intra-patient or inter-patient variability. This study will likely not happen due to the risk of infection during aspiration of TKA outweighing the potential benefits of the study.

The third limitation of the present study, as discussed earlier, is the selection bias introduced by evaluating only fluid from failed prostheses (*i.e.*, revision TKA). It would be useful to also examine the joint fluid from successful TKA, (*i.e.*, autopsy). This would be a whole new study with its own set of challenges, but it may eventually be worth pursuing. A further ascertainment bias in viscoelastic properties may have arisen from the initial protocols. At first, it appeared that f_c and G_c alone could be used to characterize the samples. Thus, when it was clear that a sample did not exhibit crossover within the measured range, experimentation on the sample was halted. It soon became clear that other information would be necessary to characterize the viscoelastic properties of these fluids, so the protocol was changed, but not all samples were still present in sufficient quantity to perform a complete battery of tests. This ascertainment bias may have contributed to the lack of a distinction between groups based upon viscoelastic properties despite a strong correlation to viscous properties that did show differences between groups.

This study was also limited by measurement error. The CSL 500 is an ancient device that is not really capable of measuring in the low shear rate range required for these experiments. It was the only rheometer available for use with biological fluids, however, so it was employed. Because of the wide inherent variability of the samples, the error had no significant effect on the results. Future work would benefit from using a newer device with a greater shear stress range, if one is available.

In addition to these limitations, there is one aspect of the study that should have been conducted differently. If I were repeating these experiments, I would utilize clearer, more meaningful means to characterize the fluid samples. These fluids were highly varied in appearance and non-homogeneous. It was difficult, in a few words or a category, to clearly express the nature of the fluid. For this reason, I think, there are no conclusions that can be drawn with regard to the gross description of the fluid. There may have been significance in the gross appearance missed by this characterization. Second, the samples were quite non-homogeneous, and since they were so varied in properties, an excellent means of dispensing one sample may be poor for dispensing another. Specifically, the pipette method used may have favored collecting less viscous portions of some samples. It would have been useful to employ a mixing protocol to improve the homogeneity of the sample before dispensing into the rheometer fixture.

3.7 Conclusions and Relevance

To summarize, flow properties of joint fluid from revision TKA were degenerate (*i.e.*, less viscous) compared to those at primary TKA, but that difference was small relative to the overwhelming variability in each group. Samples from both groups tended to exhibit properties similar to samples from historical control OA patients. Table 3.5.4 provides a useful summary of the range of properties measured. All samples exhibited some measure of shear-thinning, and most samples could be fit to the Cross model of shear-thinning. Some samples exhibited thixotropy; for these samples, steady shear viscosity could not be obtained for low shear rates, so the Cross model was inadequate.

The current study does not address whether these flow properties have relevance in TJA. Typically, as discussed in Chapter 2, boundary or mixed lubrication are considered dominant in metal-on-PE TJA, especially in TKA. Nonetheless, even if fluid film lubrication does not occur *per se*, there are occasions in which joint fluid can provide protection for the PE surface. Specifically, much damage can occur during rapid, high stress actions, such as in jumping, climbing stairs, or bracing oneself while falling. During these actions, the separation achieved when load is removed allows fluid to enter the gap between surfaces. When reloaded, squeeze film lubrication could prevent contact for a short time, reducing the impact to the PE surface. It is upon these rapid contacts that the highest stresses are applied to PE surfaces. These may lead to damage to the PE surface. Since the flow properties would describe the fluid's ability to enter the gap between surfaces, and then to maintain it once present, these properties could have substantial influence over PE damage, and ultimately clinical outcome.

Furthermore, these properties are relevant in a discussion of alternate bearing surfaces. As discussed in Chapter 2, there is evidence that hard-on-hard THA may employ fluid film lubrication; these properties are quite relevant in the design of these joints as well as potential soft-on-soft designs employing EHD lubrication. In these cases, one would be best served to consider the full range of fluid properties that have been demonstrated here. If one expects to implant a particular design into all patients, the design must perform well even when the joint fluid is least suited to good lubrication.

3.8 References

A portion of this work has been published previously,[31] though substantial new data are included in this chapter.

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CHAPTER 4

COMPOSITION OF JOINT FLUID

The protein, phospholipid, and HA contents of joint fluid samples were determined in specimens obtained from OA patients undergoing TKA and revision TKA. It was hypothesized that these components would vary widely among patients undergoing TKA, and that the composition of joint fluid in patients undergoing revision would differ from that in patients undergoing primary arthroplasty. It was further hypothesized that HA concentration and molecular weight would principally determine the flow properties previously reported. Biochemical assays were used to assess protein and phospholipid content, and size exclusion chromatography was used to determine HA concentration and molecular weight. Sixty samples were included in the study. HA, protein, and phospholipid concentrations all varied widely in patients undergoing index TKA and revision TKA. HA concentration was reduced in patients undergoing revision arthroplasty due to wear-related failure compared to patients undergoing the index procedure (0.9 ± 0.4 mg/ml versus 1.3 ± 0.5 mg/ml, mean $\pm$ standard deviation, $p = 0.04$). Other components were not different between the groups. Flow properties at high shear rates were correlated with HA concentration and, to a lesser extent, HA molecular weight, but neither protein nor phospholipid concentration. The composition of joint fluid is highly variable in the context of arthroplasty. Much of the variation in flow properties, especially at high shear rate, is explained by large variation in HA concentration and small variation in HA molecular weight. The variation in composition and lower HA concentration in joints necessitating revision may relate to variation in arthroplasty lubrication leading to highly variable wear rates and clinical outcomes.

This work serves as a foundation for future investigations of the association of joint fluid composition and properties and the tribological performance of total joint arthroplasty.

4.1 Introduction and Objectives

As discussed in Chapter 2, both boundary and fluid film lubrication are likely relevant in the tribology of total joint replacement prostheses as they are in the natural joint, though the relative contribution of these lubricating mechanisms to the tribology of TJA may depend on the implant materials and geometry of articulation. Chapter 3 examined joint fluid flow properties as they relate to fluid film lubrication. This chapter appraises the composition of joint fluid as it may relate to boundary lubrication.

Boundary lubrication depends on the adsorption of individual components of the fluid to the articulating surfaces[1] and therefore depends on the presence of molecules capable of binding to the surface. As described in Chapter 2, certain potential boundary lubricants in joint fluid (*e.g.*, phospholipids[2,3] and some proteins[4,5]) are regulated by filtration of serum-derived interstitial fluid through the joint capsule (*i.e.*, the synovial membrane plus the underlying connective tissue). Other potential boundary lubricants, such as lubricin[4,6] and superficial zone protein,[7] are synthesized by synoviocytes and articular chondrocytes, respectively. These two molecules, which are likely of the same molecular family,[8] bind to the surface of articular cartilage to provide boundary lubrication; they may perform a similar function in some prosthetic articulations.

Fluid film lubrication generally depends on the bulk properties of the fluid. The flow properties of joint fluid in the context of TKA were reported in the previous Chapter and are believed to be related to the hyaluronic acid (HA) content of the fluid;[9] HA in joint fluid is synthesized by synoviocytes. Although interactions have been shown between HA and other molecules in synovial fluid,[10-15] it is not known how these might affect boundary or fluid film lubrication.

Laboratory testing of metal-on-PE articulations has demonstrated the profound effect of the make-up of the lubricant on wear; this is underscored when comparing conditions employing no lubricant, water, and bovine serum. It seems likely that there would be some connection between the composition of joint fluid and the tribology of joint replacement prostheses *in vivo*. Despite the uncertainty regarding the determinants of the tribology of TKA, no study has evaluated the composition of joint fluid in this context.

4.1.1 Specific Aims and Hypotheses

The objectives of this study were to evaluate the content of protein, phospholipid, and HA in OA patients undergoing TKA and revision arthroplasty, and to determine which, if any, of these components correlate with selected mechanical properties of joint fluid as presented in Chapter 3. A separate group of samples from non-arthroplasty patients with effusion was evaluated as well. First, it was hypothesized that the composition of joint fluid would vary widely among patients undergoing revision TKA, and that the composition of joint fluid from revision patients would differ from patients undergoing the index procedure as well as from an historical control population of healthy individuals. This hypothesis would support a connection between joint fluid composition and variability of wear in TJA. Second, it was hypothesized that there would be a positive correlation between protein and phospholipid concentrations, and that HA would not correlate with either protein or phospholipid. The rationale for this

hypothesis relates to the sources of these joint fluid constituents: serum filtrate for protein and phospholipid, and synovial cells for HA. Third, it was hypothesized that the flow properties would correlate with the concentration and molecular weight distribution of HA as previously considered,[9] but not with the protein or phospholipid concentration, in OA patients undergoing TKA. It was further postulated that this correlation, if present, would differ in the revision group.

4.2 Materials

One hundred eight joint fluid samples were obtained from the knees of patients with joint disease. Seventy-seven specimens were from patients undergoing TKA (one due to post-traumatic arthritis, and the remainder due to osteoarthritis). Twenty joint fluid samples were obtained during revision TKA in other patients (all of these patients except one had undergone TKA due to osteoarthritis; the other individual had the diagnosis of post-traumatic arthritis). Three joint fluid samples were aspirated from effused joints that had previously undergone TKA. Seven synovial fluid samples were aspirated from joints with effusion. One sample was obtained during revision of a unicompartmental TKA that had required revision failed due to PE wear. All samples were obtained from Baw Beese Sports Medicine and Joint Care, Brigham and Women's Hospital, New England Baptist Hospital, or Massachusetts General Hospital in accordance with protocols approved by the respective Institutional Review Boards. Samples were categorized by gross appearance as normal, non-inflammatory, inflammatory, septic, hemarthrotic, or bloody, in accordance with clinical characterization of synovial fluid aspirates.[16]

Nineteen synovial fluid samples from TKA, seven samples from revision, and one effusion sample surgery were only partially examined because there was insufficient fluid available for both biochemical assay and mechanical testing (Chapter 3). Thirty-four synovial fluid samples from TKA, six samples from revision surgery, and four effusion samples were excluded from the study for the same reason. This left twenty-four index cases, seven revision cases, and two cases at effusion for which flow properties and all components were determined. Patients ranged from 37 to 89 years old, with an average age of 68 years. Of the fourteen joint fluids from revision TKA whose composition was measured, ten had undergone revision because of wear-related osteolysis and four because of mechanical problems not specifically related to wear. Patient information was obtained from medical records. See Appendix A for patient summaries.

The standard lubricant employed for laboratory wear testing, bovine serum, was also tested in this study. All bovine serum samples came from Life Technologies (Carlsbad, California) calf serum lot number 1023609, with 73 mg/ml total protein, diluted to 40% by volume in distilled water.

4.3 Methods

4.3.1 Protein Measurement

A standard curve for protein concentration (Appendix I) was prepared using bovine serum albumin stock solution 1.5 mg/ml diluted tenfold in deionized water and the Bio-Rad Protein Assay Dye (Bio-Rad, Randolph, MA). Twenty μl of synovial fluid

was diluted in the ratio 1:79 in deionized water and mixed well. Twenty µl from each diluted sample was added into a plastic cuvette containing 1580 µl deionized water and 400 µl dye and mixed well. The mixture was incubated at room temperature for five minutes and the cuvette placed into an LKB Biochrom Ultrospec 4050 spectrophotometer operating at 595 nm. The optical density reading was interpolated from the standard curve to obtain the concentration of protein in each sample. When the optical density did not fall within the standard curve, the experiment was repeated after further dilution in deionized water.

4.3.2 Phospholipid Measurement

For phospholipid concentration, a standard curve was prepared (Appendix I) using Phospholipids B Standard Solution (Wako Chemicals, Richmond, VA) and Phospholipids B Color Reagent (Wako Chemicals, Richmond, VA). Fifty µl of the synovial fluid sample was pipetted into a plastic cuvette, followed by 1000 µl Phospholipids B Color Reagent[17] and 1950 µl deionized water. The solution was mixed well and incubated at 37°C for ten minutes. The cuvette was then placed into an LKB Biochrom Ultrospec 4050 spectrophotometer operating at 505 nm. The optical density reading was interpolated from the standard curve to obtain the concentration of phospholipids in each sample.

4.3.3 Hyaluronic Acid Analysis

SEC was employed to determine the molecular weight of HA in the joint fluid samples. While SEC is the most widely used method, there have been reports using electrophoresis[18,19] or intrinsic viscosity[20] to evaluate HA molecular weight. Each fluid sample was centrifuged for ten minutes at 16,000 *g*, then diluted serially in ratios between 1:9 and 1:39 in distilled water containing 0.15 M sodium chloride. Twenty µl of 2 mg/ml pronase (catalogue number 53702, lot B34839, Calbiochem, San Diego, CA) with 3 mg/ml calcium chloride in distilled water was added to approximately 1.5 ml of diluted synovial fluid and mixed well. The mixture was incubated at 37°C for 16 hours to digest the protein in the sample.[21] This procedure served two purposes: first, it prevented interactions between protein and HA from affecting the elution time of HA; second, it reduced the overlap between the HA peak and protein peak when reading the eluted sample.

Upon removal from the water bath, samples were stored at 25°C until needed for SEC. A Waters Model 2690 Separations Module (Waters Corporation, Milford, MA) was used to inject 300 µl of each sample into a Waters Ultrahydrogel Linear 10 µm 7.8 x 300 column (catalog number WAT011545, Waters Corporation, Milford, MA) at a rate of 0.8 ml/min with a mobile phase of 0.15 M sodium nitrate. Joint fluid samples were ultrafiltrated prior to entering the column.

At first, an ultraviolet light detector operating at 206 nm was used to measure HA after separation by SEC.[22] Using this method of detection, we found it impossible to separate proteins from HA, even when mobile phase and flow rate were varied over a wide range. Refractive index was found to be a more appropriate detection technique for this application, particularly because peaks were easier to distinguish. Elution of HA and other species were measured, therefore, using a Waters 410 Differential Refractive Index

Detector (Waters Corporation, Milford, MA). To ensure that proteins did not elute simultaneously, optical density at 260 nm and 280 nm were measured on a Waters 2487 Dual Wavelength Absorbance Detector (Waters Corporation, Milford, MA). Data were collected using Millennium software (Waters Corporation, Milford, MA), and exported to spreadsheet for analysis. Samples were serially diluted to as low as 1:199 and run again to reduce non-Newtonian viscous effects. All samples were run in duplicate at the final concentration.

A standard curve of HA molecular weight versus elution time was generated using sodium hyaluronate standards 0.768 MDa, 1.26 MDa (part number 100-005, lot number 02-061), and 1.68 MDa (part number 4876-04, lot number 871576, Genzyme, Cambridge, MA). Viscosity average molecular weight (M_v) of the standards was determined using intrinsic viscosity. The elution time of peak refractive index was considered the elution time for a given molecular weight. A linear relationship between logarithm of M_v and time was determined. Although the highest molecular weight standard used to generate this curve was 1.7 MDa, previous HA calibrations on the same column have demonstrated a linear relationship up to a molecular weight of 2.0 MDa.

Baselines were calculated using two points outside the range of the peaks or, in the case of overlapping peaks, at the point of relative minimum between peaks. Using the established relationship between HA molecular weight and time, the peak molecular weight (M_p) was calculated using the elution time of the point of maximum absorbance in the peak. Further, the number average (M_n), weight average (M_w), and z-average (M_z) molecular weights were calculated for each sample. For these calculations, it was assumed that the incremental refractive index at a given elution time was proportional to the total *mass* of HA at the molecular weight corresponding to the elution time.

Total mass of HA in each sample was determined from the total area under the refractive index curve. The validity of this method of measurement was confirmed by comparison between this method and the carbazole reaction, described by Dische and Rothchild.[23] The carbazole reaction was performed on aliquots of three standards and one joint fluid sample.

4.3.4 Correlation between Joint Fluid Composition and Flow Properties

HA concentration and molecular weight as well as protein and phospholipid content were correlated to viscous and viscoelastic properties of the same samples, as determined in Chapter 3. The viscous properties evaluated included: η_{1Pa}, the steady shear viscosity at 1 Pa shear stress; η_0, the limiting steady shear viscosity at low shear rate; d, the rate index, which describes the relationship between shear rate and viscosity in the shear-thinning region; and c, which is the reciprocal of the shear rate at which shear thinning begins. The viscoelastic properties G' and G'', storage and loss modulus, were evaluated through a range of physiological frequencies. When storage modulus exceeded loss modulus at high frequencies, f_c, the crossover frequency, and G_c, the modulus at crossover, were evaluated as well. Viscous properties, protein, phospholipid, and HA content were all measured in twenty-four samples from index TKA and seven samples from revision TKA. In other samples, some, but not all, of these components and properties were measured.

4.3.5 Statistical Methods

The experiments were designed to find, with 95% confidence (*i.e.*, $\alpha = 0.05$ and $\beta = 0.05$), a 20% difference between groups in each component, assuming 15% coefficient of variation. This required a sample size of ten for each group.[24] This sample size was achieved for protein and phospholipid, but the sample size obtained for HA was only sufficient to find differences of 25% or more. Comparisons between mean concentrations or molecular weights were performed using ANOVA. Simple regression analysis was performed on individual components and on flow properties to correlate composition and properties.

4.4 Determination of Methods

The method used to determine protein concentration, commonly known as the Bradford assay, pervades the biological sciences and is often performed in our lab as part of the Western blot procedure. The method used to determine phospholipid concentration, while less widely used, is also well-described and commercially available. Likewise, the carbazole reaction is well established as a means to determine HA concentration in joint fluid. Since these techniques are fully described in the literature, only the methodology used to determine HA molecular weight is discussed in detail here.

4.4.1 Storage and Handling of Joint Fluid

Since assays were performed long after samples were obtained, samples were stored at -70°C. When sufficient fluid existed, samples were separated into three microcentrifuge tubes for storage. Prior to biochemical assay, only the aliquots needed for a particular assay were thawed at room temperature. Since HA evaluation occurred many miles from the storage facility, these samples were kept cold with ice packs while transported. This protocol differs from that employed in Chapter 3 because rheological experiments were performed a short distance from the storage facility.

4.4.2 HA Molecular Weight by the Saari Protocol

The use of an SEC solvent delivery system and detector were kindly donated by EIC Laboratories (Norwood, MA), though technical expertise was not available. Since the eluent detector available at EIC was ultraviolet absorption, a protocol was used that employed ultraviolet absorption alone for measurement of eluent. An extended effort was made to measure HA molecular weight and concentration in joint fluid using modifications to the protocol of Saari *et al.*[22] These authors used ultraviolet absorption at 206 nm in conjunction with SEC to evaluate HA in synovial fluid without pretreatment or dilution. Because the same authors later used this method to evaluate HA molecular weight from THA,[25] it was anticipated that this method could be used to determine HA molecular weight and concentration in TKA with little modification.

Size exclusion chromatography was performed using a Dynamax Solvent Delivery System SD-200 Pump (Varian, Walnut Creek, CA) equipped with an Aquagel-OH 60 15μm column and an Aquagel-OH 40 15μm column (Polymer Laboratories, Amherst, MA) in series. These columns were not the same as those used by Saari *et al.*, but were newer columns designed specifically to separate HA. Eluent was monitored and recorded by a Dynamax UV-1 Variable Wavelength UV/Visible Absorbance Detector

(Varian, Walnut Creek, CA) operating at 206 nm, consistent with the Saari protocol. All measurements were performed at room temperature.

Before being injected into the column, samples were centrifuged on an Eppendorf 5415 D Centrifuge for five minutes at 16,100 *g* to remove cellular debris. Based upon the Saari protocol, the initial elution buffer was 50 mM sodium (3) phosphate (Na_3PO_4) in HPLC quality aqueous solution, titrated to pH 6.5 with sodium hydroxide (NaOH). The pump delivered solvent at a flow rate of 1.0 ml/min. Twenty microliters of sample were injected into the solvent flow.

HA standards eluted with excellent separation using this protocol: a linear relationship was determined between the logarithm of HA molecular weight and elution time. A pilot study of three joint fluid samples was not as promising, however. Joint fluid samples exhibited substantial overlap between HA and protein peaks; the protein peak dwarfed the HA peak by an order of magnitude. To measure HA molecular weight and concentration, it is essential to obtain full separation of molecular species. This initial protocol achieved insufficient separation for two joint fluid samples, and achieved virtually no separation in the third.

Modifications to Saari

Therefore, over the next two months, modifications to several aspects of this protocol were attempted. For each condition, tests were performed on the worst performer of the three joint fluid samples tested under the Saari protocol (Study ID 003). In no case did separation between HA and protein peaks improve. First, flow rate was modified, using 0.5 ml/min and 2 ml/min. At low flow rates, diffusive processes flatten out the peaks, decreasing the output measurement for each unit time. At high flow rates, small molecules that typically enter pores in the column and therefore elute more slowly than large molecules may flow past the pores, thus reducing the separation between species of different molecular weight. The rationale behind changing the flow rate is to optimize signal to noise ratio at an intermediate flow rate. Unfortunately, it appeared that 1.0 ml/min was close to this optimal point, since changing the flow rate in either direction *reduced* the separation between peaks.

Second, each column was used independently. Since the columns are designed for different molecular weights, one of the columns may perform all of the relevant separation. The other column, if not separating molecules, may merely increase opportunity for diffusion, reducing the output signal relative to noise. Therefore, a flow rate of 0.5, 1.0, and 2.0 ml/min was attempted for each column by itself. No improvement in peak separation was found under any combination of flow rate and column.

Next, pH was modified, first to 8.0, then to 4.0. Since conformation and binding affinities of HA and proteins depend strongly on pH, modifying this parameter could improve the performance of the SEC column in separating these molecules. Using the two column system at both 1.0 and 0.25 ml/min under these two conditions of pH brought about no improvement in separation between the molecular species.

Finally, the buffer was modified on the suggestion of Polymer Laboratories (column manufacturers). Then the buffer was changed to 0.2 M sodium nitrate ($NaNO_3$) plus 10 mM sodium (1) phosphate (NaH_2PO_4), but it was no improvement. In fact, the buffer actually masked any measurement because of high absorption by nitrate at 206 nm.

The process of trial and error, while fruitless in this case, is often used in conjunction with SEC, since the mechanics behind molecular separation are not completely understood. The only additional change that could have been made to imitate the apparently successful methodology of Saari would have been to change columns. Although the columns employed were not the same as those used by Saari *et al.*, the columns were clearly capable of separating HA based upon molecular weight, as demonstrated by HA standard elution times. Thus, the columns were likely as good as or better than those of Saari, and no improvement in separation technique could be made. An alternative method to measure HA without measuring protein was necessary. This could be accomplished either by changing the detector (to not measure protein) or by eliminating the protein itself.

Addition of Protease

Since numerous workers had shown reference to interactions between HA and proteins (see section 2.2.5), there was reason to believe that these interactions could affect the behavior of HA in the column. In particular, a protein-HA complex may behave like a larger molecule than HA by itself. Thus, one might overestimate the molecular weight of HA in joint fluid if protein is not removed. As discussed in Chapter 2, at least one group has used protease to digest protein prior to using SEC.[26]

After a six month sabbatical from this aspect of the thesis, experiments were resumed. To remove the protein peak, diluted samples were maintained in a 56°C water bath for at least 15 hours with 1 mg/ml proteinase K solution. Samples were then centrifuged on an Eppendorf 5415 D Centrifuge for five minutes at 16,100 times the acceleration of gravity. Experiments on bovine serum with and without protease demonstrated the efficacy of protease in removing the protein peak. Experiments on HA standards demonstrated that protease effected HA elution time minimally. Additional improvement in separation was achieved by diluting samples substantially and increasing the injection volume tenfold. Using a final buffer of 150 mM NaCl and 56 mM sodium (3) phosphate (Na_3PO_4) aqueous solution buffered to pH 7.4, the apparent HA molecular weight and concentration of many joint fluid samples was measured.

Further Complications

Unfortunately, before the experiments could be completed, a power failure destroyed the electronics of the pump. Since communication between pump and detector were necessary for accurate measurement of elution time, the system became inoperable. After extensive resuscitative efforts, it was necessary to abandon work at EIC. Although it was a painful conclusion to these experiments, significant progress had been made in methodological development. Specifically, the need for joint fluid dilution and protein digestion were both established. Furthermore, the software used to analyze the detector output was cumbersome, and would not have permitted important aspects of data analysis. Consequently, it was perhaps better that the experiments had to be repeated elsewhere.

A few additional lessons were elucidated by these experiments (in addition to the importance of protein digestion and joint fluid dilution). First, ultraviolet absorption is a poor method to distinguish HA from protein. Since protein absorbs so much light at 206 nm, even a small tail of a protein peak interferes with the HA peak. Second, use of a

single detector does not help confirm the identity of the species measured. Therefore, even if the HA peak can be fully isolated, one cannot demonstrate the peak is HA and not some high molecular weight protein or a protein-HA complex.

Revisiting the work of Saari *et al.*, it is perplexing that they could make their measurements without first digesting the protein. The figures presented in publication, referred to as "typical," all displayed substantial protein-HA overlap. Perhaps the authors downplayed the difficulties associated with the methodology. These difficulties may be exacerbated by low HA concentration and molecular weight, and large amounts of high molecular weight proteins – all conditions likely in joint fluid samples from TKA, as the present experimental results show. Thus, though the Saari protocol may be acceptable for certain cases, it was not acceptable for the present study.

4.4.3 HA Molecular Weight by the Hyaluron Protocol

Soon thereafter, Hyaluron, a manufacturer of HA, agreed to lend their equipment and expertise for our benefit in HA measurement from joint fluid. Having more options for a detection device than at EIC, I chose (on Hyaluron's recommendation) to use refractive index to measure eluent and ultraviolet absorption at 260 and 280 nm to confirm the absence of protein. The pump, column, and solute were chosen based upon the expertise of Hyaluron as well. These were not modified through the course of experimentation, and are described in section 4.3.3.

Initially, HA standards were eluted through the column, and detected nicely using refractive index. These standards were obtained commercially, and their molecular weights confirmed by intrinsic viscosity measurement. Initially, the same samples that performed poorly in the previous apparatus (Study ID 003 and Study ID B) were injected into the present one, diluted tenfold. One of the output curves generated from this injection is given in Figure 4.4.1.

With this output, there were two problems. First, the presence of protein peak interfered with the measurement of HA. As shown in Fig. 4.4.1, the magnitude of the protein peak resulted in interference with the HA peak, obscuring the true amount of HA present. This was confirmed by ultraviolet absorbance at 260 and 280 nm (not shown), in which protein began to elute before HA elution (via refractive index) was completed. These findings supported previous work (section 4.4.2) and work by others[26,27] suggesting that it was necessary to remove the interaction between proteins and HA prior to SEC. This was performed using proteolytic degradation[21] and confirmed by measuring absorbance at 260 and 280 nm. In all cases, there was no ultraviolet absorbance at these two wavelengths within the relevant range of elution times after proteolytic degradation (see Fig 4.4.2, below). A peak remained at elution times greater than 9 minutes in both ultraviolet absorption and refractive index, corresponding to molecules of size less than 100 kDa.

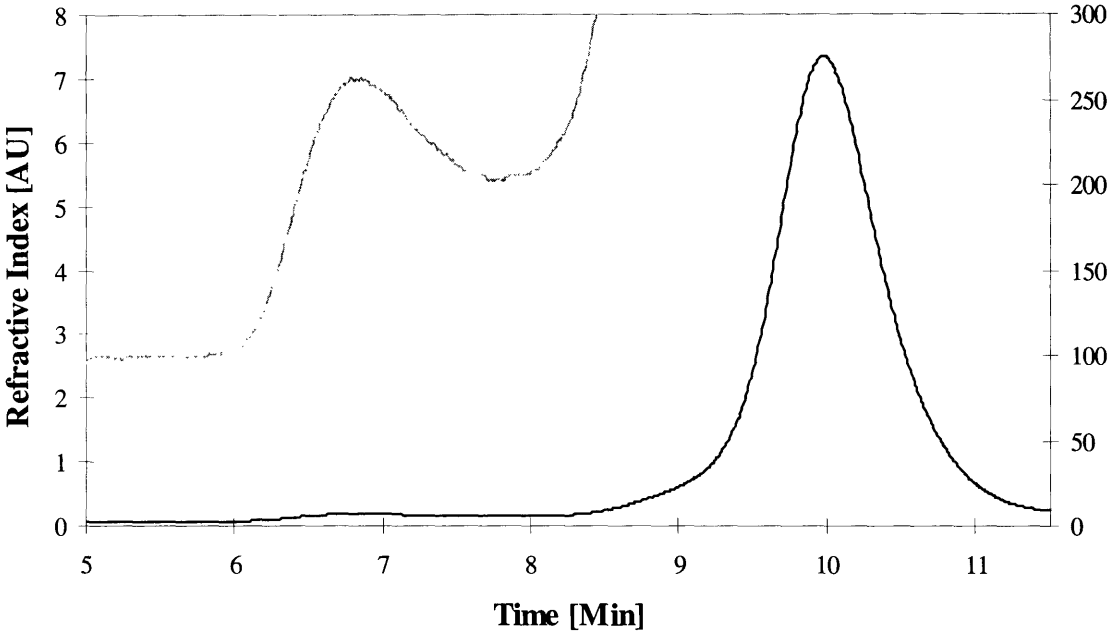

Fig 4.4.1 Output curve from a joint fluid sample (Study ID B) diluted 1:9 The black line represents the complete curve of refractive index versus time using the scale to the right. The gray line represents the *same* data using the detailed scale on the left. The first peak, barely visible on a large scale, represents elution of HA. The second, much larger peak represents elution of proteins. It is clear that although the protein peak elutes well after the HA peak, its overwhelming size results in some overlap between the peaks, as seen on the detailed scale (to the left).

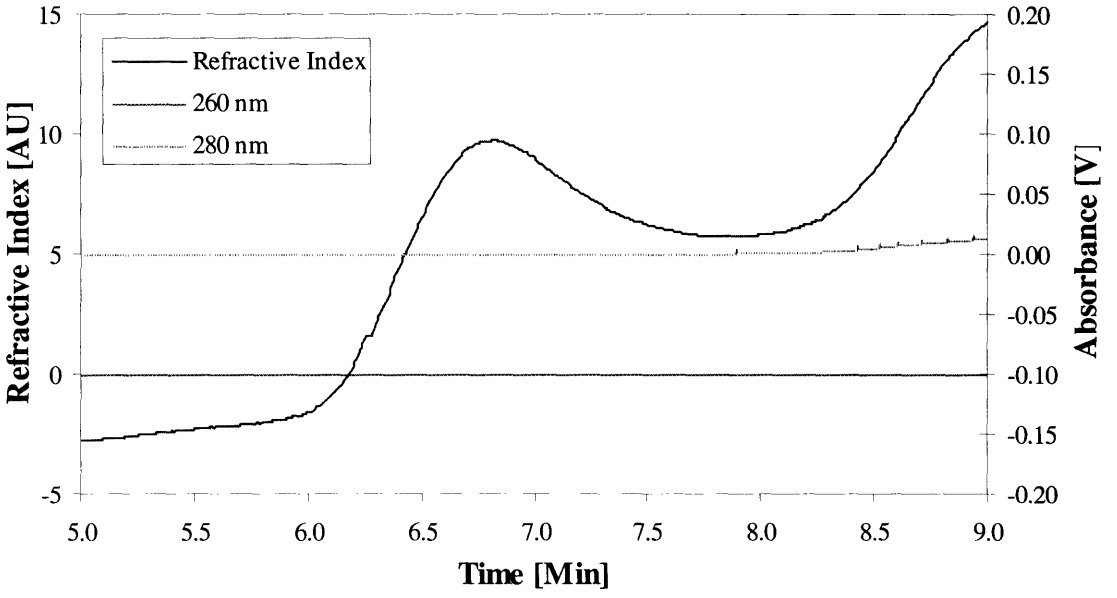

Fig 4.4.2 Refractive index and ultraviolet absorption after digestion with proteinase Lines representing refractive index and ultraviolet absorption show that digestion with proteinase removes high molecular weight protein-HA interactions. There is virtually no ultraviolet absorption within this range for this sample (Study ID 003). "Viscous fingering" is evident in the double HA peak.

The second problem with these initial efforts was that the output curve was affected unpredictably by the high viscosity of the eluent, in a phenomenon known as "viscous fingering."[28] This effect can be demonstrated by inconsistency in elution time and output shape as the sample is diluted. Its presence was variably manifest, but is evident in the double HA peak above in Fig 4.4.2. To minimize this effect, and to demonstrate its minimization, samples were run at approximate dilutions of 1:9, 1:19, and 1:39. Using this protocol, it was evident that HA retention time depended on concentration at concentrations greater than 20 μg/ml. At high concentrations, the distribution of elution time (reflecting the apparent molecular weight distribution) was not bell-shaped, and depended on concentration. These characteristics indicate that non-Newtonian viscous effects (*i.e.*, "viscous fingering") were interfering with molecular weight determination (Fig 4.4.3, light gray and dark gray lines). Consequently, samples were diluted serially in 150 mM sodium chloride and mixed well until the area under the refractive index curve indicated a final concentration less than 20 μg/ml. At this concentration, the curve of absorption versus elution time followed a typical polymeric distribution in all samples, and did not depend on concentration (Fig. 4.4.3, black lines). Furthermore, the dilution of joint fluid reduced the overlap between the protein peak and the HA peak, increasing the accuracy of the measurement of both HA concentration and molecular weight distribution. It is interesting to note that, unlike joint fluid samples, HA standards did not exhibit "viscous fingering" even at concentrations as high as 100 μg/ml.

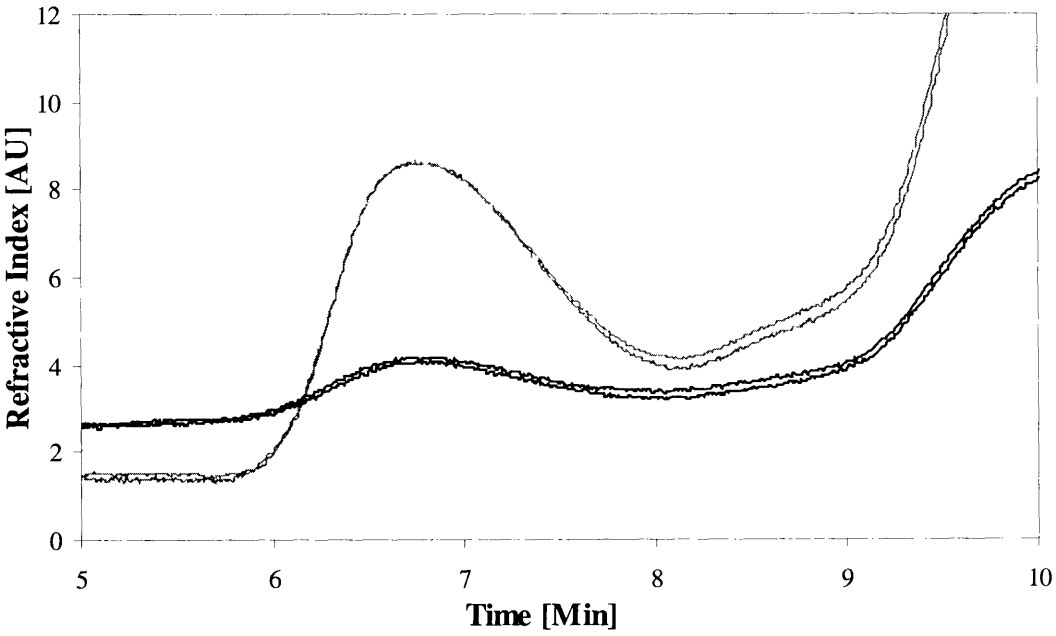

Figure 4.4.3 Refractive index measurement for joint fluid from the left knee of a 70 year old woman at TKA (Study ID H21) Light gray lines represent 19:1 dilution. Dark gray lines represent 39:1 dilution. Black lines represent 199:1 dilution. Each test was performed in duplicate, with both injections shown. Final concentration ~ 5 μg/ml.

Even at this low concentration, it was not possible to completely separate the HA and protein peaks in some cases. This effect can be seen above in Fig. 4.4.3 or below in Fig 4.4.4, which is a magnified view of one of the 1:199 dilutions from Fig. 4.4.3. For this sample, absorption did not return to the baseline between the HA and protein peaks. To most accurately estimate the HA concentration at each molecular weight, a line was drawn, by inspection, tangent to the left and right bases of the HA peak. Using the baseline correction, an elution peak in the shape of a normal distribution was generated for each sample (Fig. 4.4.5). Refractive index at a given elution time were converted to mass at a given molecular weight according to calculations given in Appendix J. The molecular weight corresponding to the highest excursion of refractive index above the calculated baseline was determined as M_p. M_n, M_w, and M_z were also calculated for each peak, and polydispersity was calculated as the ratio of M_w to M_n. See Appendix K for a complete description of the calculation of molecular weight parameters.

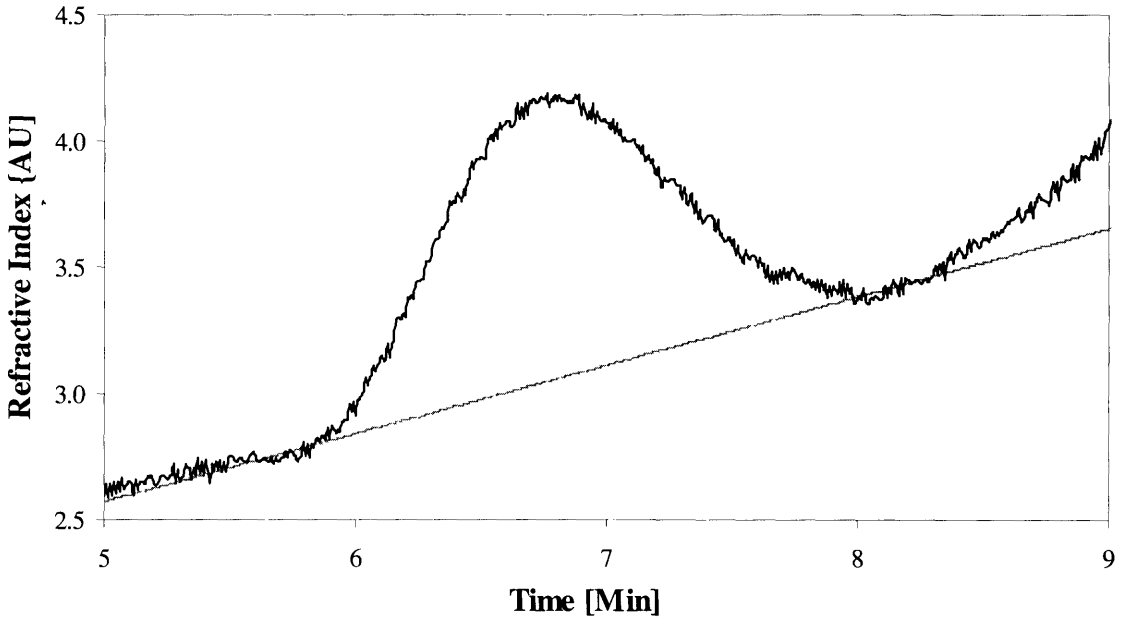

Figure 4.4.4 Baseline calculation for the same case as Fig. 4.4.3 (Study ID H21) The curve indicates absorbance at 199:1 dilution. The baseline has been defined by the line tangent to the depression prior to and subsequent to the HA peak. Absorbance due to the HA peak is recalculated based on this baseline.

With the addition of proteinase according to the protocol described in section 4.3.3, diluted samples were eluted serially. HA standards (also treated with proteinase) were interspersed with the samples to verify the column's ability to separate molecules by molecular weight.

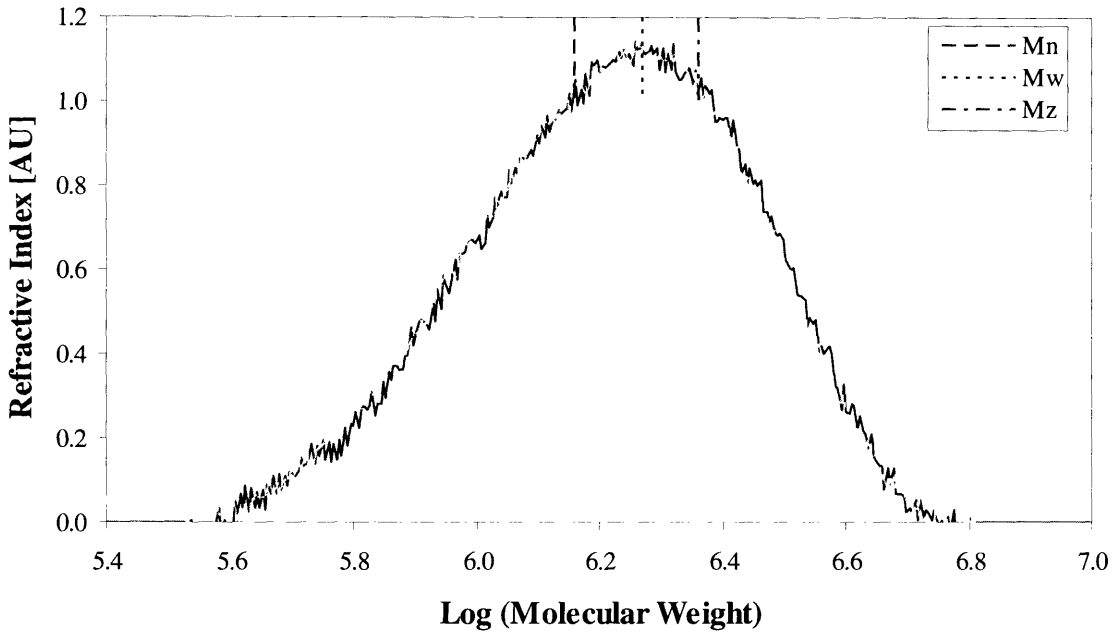

Figure 4.4.5 Histogram determined from the sample in Fig. 4.4.3 Both injections at 199:1 dilution are shown. For reference, M_n, M_w, and M_z are labeled. Units of the abscissa are left off for simplicity.

4.4.4 Conclusions about HA Molecular Weight Determination

Based upon the experience of these experiments, it is clear that both dilution and protein digestion are essential components of successful HA molecular weight determination by SEC. Furthermore, refractive index appears to be superior to ultraviolet absorption because proteins may absorb more ultraviolet light than HA. It is preferable to use an Aquagel-OH 60 15μm column and an Aquagel-OH 40 15μm column (Polymer Laboratories, Amherst, MA) in series. Although equivalent columns may exist, these columns appeared to perform better than the column used in the Hyaluron protocol in separating high molecular weight standards (see Appendix J). Finally, given the resources to use any methodology at all, there are several other means to determine HA molecular weight in joint fluid.[29-31] These methods may be easier and/or more reliable than the present methodology. In the current project, however, resources were limited, and the protocol chosen adequately determined HA concentration and molecular weight in this group of joint fluid samples.

4.5 Results

The amount of fluid obtained from the joints varied widely. In index TKA, the mean amount obtained was 7.0 ml (standard deviation: 4.3 ml, range: 1.5 to 19 ml); in revision, the mean amount was 6.4 ml (standard deviation: 6.2 ml, range: 1.5 to 22 ml). There were cases in both groups in which no fluid could be obtained – these are not included in this analysis. Joint fluid samples obtained at revision TKA grossly resembled those obtained at index TKA. Both groups included all categories (based on gross examination), except that none of the samples obtained at revision were septic. A gross description of all joint fluid samples is given in Appendix F. Septic samples were not

found at revision TKA because of the inclusion criteria of the study. All other categories were represented in the same proportions, except that samples obtained at revision were more likely to be hemarthrotic (24%, compared to 14% of samples at primary TKA). This difference was not statistically significant by Fisher's exact test (cf. section 3.5).

4.5.1 Concentrations of HA, Protein, and Phospholipid

The protein, phospholipid, and HA concentrations for the joint fluid samples are given in Table 4.5.1. The range of values found for each component of joint fluid in the index and revision TKA samples was large. Protein content spanned the range of values found in healthy and diseased synovial fluid, as presented in Table 2.2.5. HA concentration was consistent with values obtained from the joint fluid of OA and RA patients. Likewise, the phospholipid concentration was consistent with values obtained from diseased joints. Insufficient data were available from healthy joints to compare with the present phospholipid data. Complete results are given in Appendix L.

Table 4.5.1: Composition of joint fluid obtained at TKA and revision TKA Summary of protein, phospholipid, and HA content in the context of the findings of others. New groups examined are "Index TKA" and "Revision TKA." Present data are given as mean ± standard deviation, and compared with historical controls (shaded data), which are given as in Table 2.2.5. Concentrations are given as mg/ml. [a]HA M_p = peak average molecular weight, MDa; [b]Data include only revisions due to wear-related failure.

Group	*Protein*	*Phospholipids*	*Hyaluronic Acid*		
			Conc.	M_p[a]	*n*
Index TKA	27 ± 10 (n = 43)	0.52 ± 0.18 (n = 41)	1.3 ± 0.5	1.9 ± 0.1	24
Revision TKA	34 ± 13 (n = 10)	0.52 ± 0.19 (n = 10)	0.9 ± 0.4[b]	1.8 ± 0.1	7
Normal	10 – 30	~ 0.1	2 – 4	~ 2 MDa	N/A
OA	24 – 44	0.1 – 0.5	0.5 – 1	2.4 – 3.2	N/A
RA	27 – 63	0.4 – 0.8	0.1 – 0.9	~ 0.6	N/A

Joint fluid obtained at revision had a 25% higher protein content than that at index TKA (ANOVA, p = 0.05). The relatively large difference in mean values between the two groups was partially masked by the wide range in results. Specifically, the coefficient of variation was quite large for each measure (except HA molecular weight). Thus, larger differences between groups were necessary to determine statistical significance. Phospholipid concentration was virtually the same for the two groups. Among samples obtained at revision surgery, protein and phospholipid concentration did not depend on whether revision was performed for wear-related reasons (n = 10) or not (n = 4). When only those revision cases that were due to wear-related failure were considered, the results were similar to those when all revision cases were considered.

HA concentration was measured in one case of revision for reasons unrelated to wear. In this case, HA concentration was threefold higher (2.8 mg/ml) than the mean value (0.9 mg/ml) for the seven cases of revision arthroplasty subsequent to wear-related failure. Excluding this case, joint fluid obtained from patients whose TKA failed due to wear had significantly lower HA concentration (30% lower) than joint fluid obtained at index TKA (Table 4.5.1; ANOVA, p = 0.04).

Relative to other parameters, the spread in HA molecular weight data was small (Table 4.5.2). The HA molecular weights in fluid samples for patients undergoing TKA and revision TKA were very similar. A small difference was detected between the two groups as measured by M_p (p = 0.05), but this was probably not meaningful. Polydispersity was the same in the two groups. This analysis was not significantly affected by discounting the sample obtained at revision for a reason unrelated to wear. Consequently, this sample was included in the analysis (unlike in Table 4.5.1).

Table 4.5.2 HA molecular weights in joint fluid from index and revision TKA Data are presented as mean ± standard deviation in MDa. M_p = peak average molecular weight; M_n = number average molecular weight; M_w = weight average molecular weight; M_z = z-average molecular weight.

Group	M_p	M_n	M_w	M_z	*polydispersity*
Index TKA (n = 24)	1.9±0.1	1.4±0.1	1.8±0.1	2.2±0.1	1.3±0.04
Revision TKA (n = 8)	1.7±0.1	1.3±0.2	1.7±0.2	2.1±0.2	1.3±0.08

4.5.2 Correlations among Concentration of Components

Among all samples, there was a positive correlation between protein and phospholipid content ($p < 0.0001$, $R^2 = 0.47$; regression analyses performed are linear regression unless otherwise specified). Within the revision group, protein and phospholipid were more highly correlated, with $R^2 = 0.57$. Upon examination of this correlation, 13 points fit a linear relationship and one outlying data point did not (Fig. 4.5.1). With this single point excluded, the correlation increased still more to $R^2 = 0.87$ ($R^2 = 0.88$ when tied to the origin).

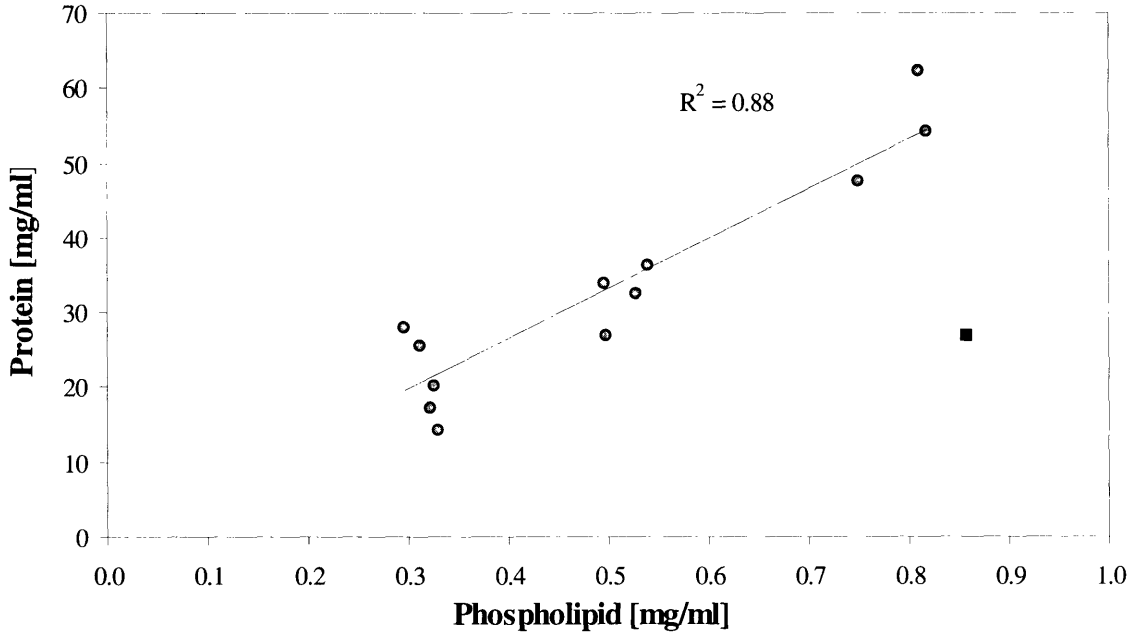

Fig. 4.5.1 Protein concentration versus phospholipid concentration in revision TKA The 13 points that fit a linear relationship are shown as gray circles. The one outlier is shown as a black square.

Both protein and phospholipid displayed a negative correlation with HA concentration (protein, $p = 0.0035$; phospholipid, $p = 0.005$). These correlations explained less than half of the variation in the data when modeled by linear regression (protein, $R^2 = 0.25$; phospholipid, $R^2 = 0.34$). When only revision cases were considered, linear correlation between phospholipid and HA content was more meaningful ($R^2 = 0.66$).

Gross appearance correlated with composition to a limited extent. Normal-appearing samples (clear to pale yellow and transparent) had high protein content (41 mg/ml) and low HA concentration (0.40 mg/ml) relative to the other groups. In the case of protein, this difference only reached statistical significance when compared to the septic group (21 mg/ml). In the case of HA, this difference reached statistical significance for both the septic group (1.4 mg/ml) and the hemarthrotic group (1.5 mg/ml). For all other gross categories, there were no differences in composition. Those samples visibly contaminated with blood ($n = 10$) – having dark red portions, as opposed to hemarthrotic samples in which the entire sample had a reddish tint – contained more protein and phospholipid than those not contaminated with blood (phospholipids: $p = 0.05$; protein: $p = 0.12$). These differences were more substantial at index TKA than at revision TKA. There was no relationship between blood contamination and HA content.

Each measure of average molecular weight correlated to all other measures of average molecular weight ($p \leq 0.0001$), with the strongest correlation between M_w and M_z (linear regression, $R^2 = 0.92$). There was a negative correlation between polydispersity and M_n ($p < 0.0001$) and M_w ($p < 0.001$), but not between polydispersity and M_p or M_z. Concentration also related to M_n ($p = 0.008$) but not to any other molecular weight parameters. None of these effects demonstrated a meaningful linear regression correlation. Finally, HA concentration related to polydispersity ($p < 0.0001$; linear regression, $R^2 = 0.60$), with higher concentration HA having a wider distribution of molecular weights.

HA molecular weight did not correlate to protein or phospholipid concentration. Both protein and phospholipid concentration related to polydispersity, but this correlation did not explain a large portion of the variability in data by linear regression (protein: $p < 0.0001$, $R^2 = 0.37$; phospholipids: $p < 0.002$, $R^2 = 0.25$). There was no difference in any molecular weight parameter based upon gender, leg, or reason for revision. There was a weak negative correlation between molecular weight of HA and age among patients undergoing index TKA, but not at revision. Patient weight appeared to have an effect on molecular weight, M_p, of HA ($p = 0.02$), however, with heavier patients having higher molecular weight HA. Statistical significance was not reached for the other molecular weight parameters.

There was no correlation between patient age and any component, either for an individual group or for all samples. Likewise, there was no difference based upon gender or leg. Patient weight and height were available for eight samples at index TKA. There was no correlation between these parameters and concentration of any component of joint fluid. There was no correlation between volume and any component of joint fluid.

4.5.3 Correlation of Composition with Flow Properties

Using regression analysis, the flow properties of joint fluid samples were correlated to their composition. There were not meaningful correlations between protein concentration and most viscous and viscoelastic parameters, including η_0, η_{1Pa}, c, f_c, G_c, G', and G''. (See Chapter 3 for a complete explanation of these parameters.) In samples obtained at revision TKA, there was an inverse relationship between protein concentration and d ($p < 0.05$; linear regression, $R^2 = 0.43$). Among the group obtained at revision TKA, phospholipid concentration exhibited a weak inverse correlation with both η_0 and η_{1Pa}. The correlation was stronger for η_0 ($p = 0.007$, exponential regression: $R^2 = 0.61$). There was a negative correlation of phospholipid concentration with d for samples in both groups. The correlation was strongest among the group of samples obtained at revision TKA ($p = 0.004$; linear regression, $R^2 = 0.66$).

Including only those samples fitting the Cross model did not affect the relationships among these parameters. Although differences were not statistically significant, there was a trend towards higher protein concentration and lower HA concentration among samples that failed to fit the Cross model compared with those that fit the Cross model.

There was a strong positive correlation between HA concentration and both d and η_{1Pa} among samples obtained at primary TKA (d: $p < 0.0001$, Fig. 4.5.2; linear regression: $R^2 = 0.63$; η_{1Pa}: $p < 0.0001$, exponential regression $R^2 = 0.76$). Although this correlation was also found for the revision group for d ($p < 0.04$, $R^2 = 0.60$), it was not observed for η_{1Pa} ($p = 0.87$, $\beta = 0.05$). None of the other viscous parameters could be correlated to HA concentration when all samples were included. When only those samples fitting the Cross model were included, HA concentration could be correlated to η_0 ($p = 0.0012$, exponential regression $R^2 = 0.40$) and c ($p = 0.0066$, exponential regression $R^2 = 0.30$). Neither f_c nor G_c could be correlated with HA concentration. There were few samples that exhibited crossover, however. There was a positive correlation of storage and loss moduli at 0.5, 2.5, and 5 Hz with HA concentration. This correlation was quite strong ($p = 0.0008$, $R^2 = 0.73$ in the worst case) for samples at primary TKA (Fig. 4.5.3). Among revision samples, the same correlation appeared to exist, but there were an insufficient number of samples to perform a separate analysis.

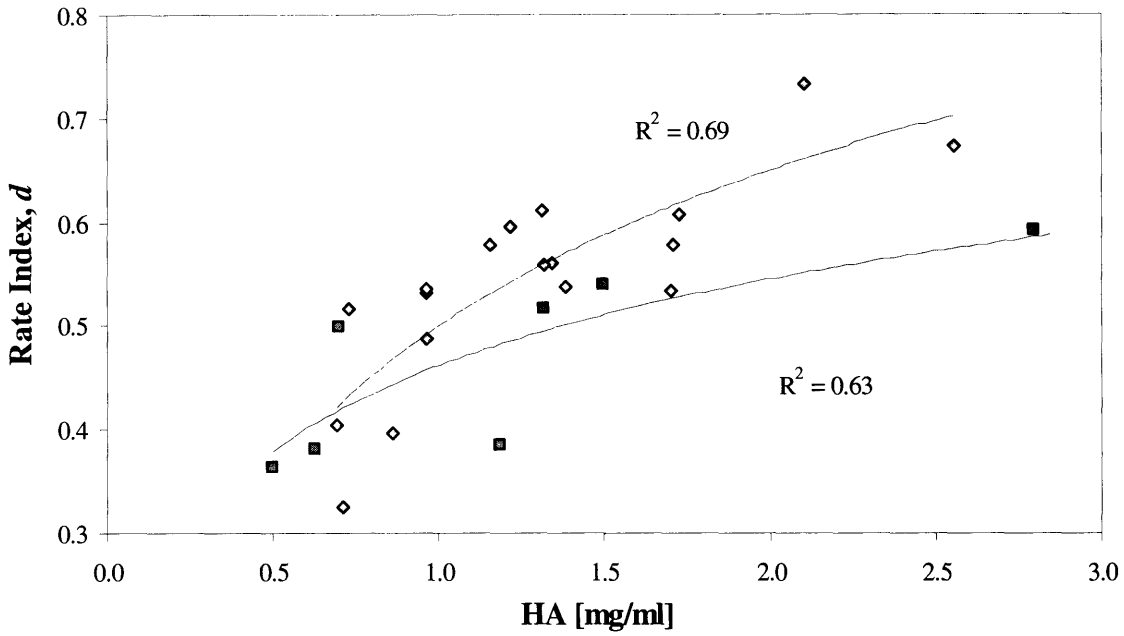

Figure 4.5.2 Correlation between rate index and HA concentration from samples obtained at primary and revision TKA Gray diamonds are samples obtained at primary TKA, and black squares are obtained at revision TKA. Best fit exponential curve and coefficients of determination are shown (power law and linear relationships may also be used; linear regression is used in text).

Considering all samples, no correlation could be found between any viscous parameter and any measure of HA molecular weight distribution. When only samples fitting the Cross model were considered, η_0 and c correlated to both M_p and M_z, but not M_n or M_w; these correlations were weak ($R^2 < 0.28$ for exponential regression). Among viscoelastic parameters, there was a strong negative correlation between f_c and M_p ($p = 0.0003$, $R^2 = 0.83$). There was also a negative correlation between crossover frequency and M_z, but much of the correlation was due to the presence of one outlying data point. Finally, there was a moderate negative correlation between G_c and M_n ($p = 0.01$, $R^2 = 0.53$). No other measure of HA molecular weight could be correlated to f_c or G_c.

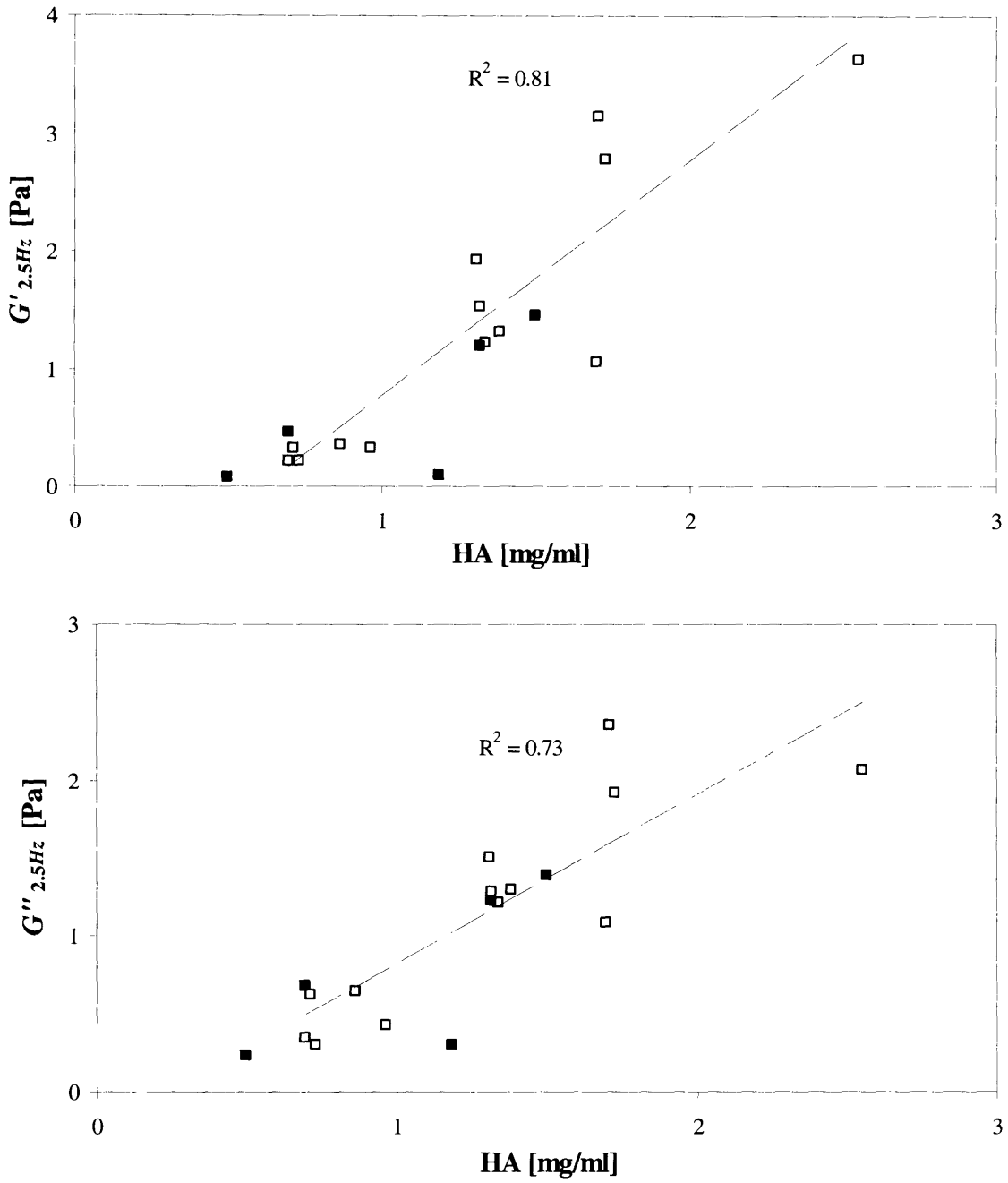

Figure 4.5.3 Storage modulus (above) and loss modulus (below) at frequency 2.5 Hz versus concentration of HA Open squares represent index TKA; closed squares represent samples from revision. Dotted line is the best fit line through the data, with coefficient of determination shown in each chart.

4.5.4 Additional Groups Evaluated

Additionally, several samples from other patient groups were evaluated (Table 4.5.3). These samples have been included primarily to boost the sample sizes for correlations between individual components and flow properties. All analyses were also

conducted with these samples excluded to prevent differences between groups to mask important relationships.

Although there were not sufficient samples obtained from these groups to make meaningful statistical analyses, certain trends were noted. Samples aspirated from effused joints had relatively low protein and phospholipid concentrations, but HA concentration and molecular weight were similar to other groups. On the other hand, among samples obtained from joints with effusion after TKA, protein and phospholipid concentrations were consistent with the other groups, but HA concentration was lower than any other sample measured. There were two cases in which TKA was performed with a diagnosis of post-traumatic arthritis. In one case, fluid was obtained at index TKA; in the other, it was obtained at revision due to wear-related failure. In both these cases, protein and phospholipid concentrations were similar to patients undergoing the same procedure under a diagnosis of OA. HA concentration was higher that the mean obtained in these groups.

The protein and phospholipid concentrations of bovine serum are also reported in Table 4.5.3. Protein concentration was similar to that reported in normal joints; phospholipid concentration was substantially higher than the concentration of most joint fluid samples.

Table 4.5.3 Composition of joint fluid from other groups and cases Concentrations are given as mg/ml. All values measured are given; the sample size is equivalent to the number of cases measured. Bovine serum data are given as mean value only. TKA Effusion = effusion after TKA; Uni-TKA Rev. = sample obtained at revision of unicompartmental TKA (Study ID 167); PTA = sample obtained from a 52 year old man undergoing TKA due to post-traumatic arthritis (Study ID H20); PTA Rev. = sample obtained from a 42 year old woman at revision TKA – the original indication for TKA was post-traumatic arthritis (Study ID 172); BS = Bovine serum

	Effusion	*TKA Effusion*	*Uni-TKA Rev.*	*PTA*	*PTA Rev.*	*BS*
Protein	22, 15	20, 34	11	31	34	21
Phospholipid	0.26, 0.31	0.29, 0.62, 0.65	0.26	0.52	0.50	0.80
HA Conc.	1.1, 1.2, 1.4	0.28	1.8	1.7	1.3	N/A
HA M_p	1.8, 1.7, 1.7	1.7	1.6	2.0	1.8	N/A
HA M_n	1.4, 1.3, 1.2	1.5	1.2	1.4	1.4	N/A
HA M_w	1.8, 1.7, 1.6	1.8	1.6	1.8	1.8	N/A
HA M_z	2.3, 2.1, 1.9	2.2	1.9	2.3	2.3	N/A

In other cases, multiple aspirations were obtained from the same patients. In two cases, joint fluid was taken from both knees undergoing bilateral TKA. In two other cases, joint fluid was obtained from one knee during TKA, and then the opposite knee in a later TKA (Table 4.5.4). In these cases, the composition of one knee did not predict the composition of the other. For example, in one case, the HA concentration in the left knee was twice that in the right. On the other hand, in another case, the phospholipid and protein concentration were very similar in the left and right knees. There were not enough such cases to make generalizations about the relationship between the function (or dysfunction) of contralateral joint capsules. The variability in joint fluid composition reflects the variability in flow properties in contralateral knees noted in Chapter 3.

In another case, joint fluid was aspirated from a knee after TKA. Revision surgery was performed three months later, at which time the joint fluid was again obtained and analyzed. In the time between aspiration and surgery, the protein and phospholipid concentration of joint fluid both increased, reflecting a decline in joint capsule function.

Table 4.5.4 Composition of multiple joint fluid samples obtained from the same patient In all but the first case, samples were obtained from different knees. Units are the same as in previous tables. Numbers in parentheses indicate study ID numbers for reference with the appendices. [a]Revision performed for wear-related failure. [b]Left TKA performed two months after right TKA. [c]Left TKA performed four months after right TKA.

Patients		*Phospholipid*	*Protein*	*HA*	M_p	M_n	M_w	M_z
69 year old ♂ After TKA[a]	Effusion (030)	0.29	20	N/A	N/A	N/A	N/A	N/A
	Revision (164)	0.31	26	N/A	N/A	N/A	N/A	N/A
52 year old ♀ Bilateral TKA	L (156)	0.32	10	2.6	2.0	1.4	1.9	2.3
	R (155)	0.40	22	1.3	2.0	1.4	1.8	2.3
68 year old ♂ Bilateral TKA	R (H07)	0.34	25	N/A	N/A	N/A	N/A	N/A
	L (H08)	0.54	29	N/A	N/A	N/A	N/A	N/A
70 year old ♀ TKA[b]	R (H12)	0.54	34	N/A	N/A	N/A	N/A	N/A
	L (H21)	0.78	45	1.0	1.8	1.4	1.9	2.3
45 year old ♀ TKA[c]	R (H03)	0.92	40	1.3	1.8	1.6	2.0	2.4
	L (H09)	0.68	33	1.7	1.9	1.5	1.9	2.3

4.6 Discussion

4.6.1 Range of Composition of Joint Fluid

The data span a wide range (factor of six for protein, more than four for phospholipid, and more than three for HA), consistent with a connection to clinical variability in tribology. As evidenced by the relatively large standard deviations for these samples, the wide spread reflects the spread of the complete data set, rather than the effects of one or a few outliers. The large range for phospholipid and protein could be relevant to the tribology of TKA, particularly with regard to boundary lubrication. Values as low as 10 mg/ml protein and 0.22 mg/ml phospholipids could be related to particularly high wear rates (outliers) observed *in vivo*,[32,33] though tribological studies are necessary to show a causal relationship between joint fluid composition and the efficacy of boundary lubrication in total joint arthroplasty. These experiments are performed in Chapter 5.

Bovine serum is generally used as the lubricant in POF and joint simulator testing of prosthetic components. Bovine serum is diluted to represent the estimated protein content of joint fluid.[34] Laboratory wear testing protocols assume that bovine serum and joint fluid lubricate similarly based upon protein concentration. If this supposition is not valid, however, the diluted bovine serum would not adequately approximate the lubricating properties of joint fluid; such could also be the case if specific proteins, which may be absent in bovine serum or in some joint fluid samples, are critical lubricants.

4.6.2 Comparison among TKA Patients, Revision Patients, and Previous Reports

As discussed in section 2.2, and shown in Tables 2.2.5 and 4.5.1, protein and HA concentration of joint fluid have been measured in several cohorts of healthy and diseased joints. Phospholipid concentration has been less often studied, particularly in healthy joints. Protein, phospholipid, and HA concentrations of joint fluid from TKA were generally consistent with previous reports from cohorts with OA, both in mean value and the spread in the data.

After arthroplasty, there were no significant changes in total protein and phospholipid. Although protein content increased, the difference did not reach statistical significance. These data rejected the hypothesis that protein and phospholipid content of joint fluid from revision patients would differ from those of patients undergoing the index procedure. It would be useful to examine changes in joint fluid composition in the same patient with time after arthroplasty. The similarities between the pre- and post-arthroplasty groups suggest a similarity between the repaired synovial membrane present after TKA and that present before TKA (as noted above), though many other factors complicate this comparison.

HA concentration was significantly reduced in the group undergoing revision for wear-related failure, suggesting an association between the two. A rationale for a causal relationship (*i.e.*, low HA concentration leading to wear) is that HA concentration dominates the flow properties of joint fluid, which in turn determine fluid film lubrication of TKA. When HA concentration does not create the necessary flow properties to generate fluid film lubrication, wear processes are accelerated, leading to wear-related failure. The small number of samples tested does not justify this conclusion, but merely suggests a possible relationship.

4.6.3 Correlations among Protein, Phospholipid, and Hyaluronic Acid Concentration

There was a meaningful positive correlation between protein concentration and phospholipid concentration in patients undergoing index TKA and in the revision group, supporting my hypothesis. The relationship between protein and phospholipid concentration was similar for the two patient groups, suggesting that the joint capsule (much of which may be removed during surgery) was reconstituted after arthroplasty, and functioned in a similar fashion pre- and post-arthroplasty. This occurred even in joint arthroplasty patients with severe enough problems to necessitate revision surgery. Although it is tempting to draw conclusions from the increased correlation between protein and phospholipid in revision surgery, I will not do so. There are not sufficient data to claim that protein and phospholipid from joint fluid in TKA are more strongly correlated than these components in synovial fluid. The variability in the data may be due to an alteration of the mechanisms responsible for maintenance of the protein and phospholipid concentrations in the joint. These alterations occur in disease as well as in reparative processes after the damage induced to the synovial membrane during TKA.

There was a negative correlation between HA concentration and both protein and phospholipid concentrations for both pre-arthroplasty knees and joints undergoing revision surgery. This finding rejected our hypothesis that HA would not correlate with either protein or phospholipid. These parameters have not previously been correlated, to my knowledge, within any particular disease state. In prior work, HA has been shown to

be associated with cells of the repaired synovial membrane after TKA,[35] suggesting that these cells manufacture HA as do type B synoviocytes in the natural joint. Collectively, these data support a connection between the function of the regenerated synovial membrane to filter joint fluid and the function of synoviocytes to produce HA. Possible explanations include a synovial membrane response to low HA concentration, allowing increased protein entry. Alternatively, high protein content in joint fluid may down-regulate HA production by synoviocytes. This supposition, while complicated by the wide variability of joint fluid volume, warrants further study.

No conclusions can be drawn regarding proteins and phospholipids versus age, gender, leg, height, or weight, except that there are no apparent correlations. This finding is consistent with previous reports in healthy patients, in which there was no change with age (in adulthood) in the concentration of HA in synovial fluid.[9]

4.6.4 Hyaluronic Acid Molecular Weight Distribution

In contrast to the concentration data, there was not a wide spread in the molecular weight of HA in the samples studied. The difference between synovial fluid at index TKA and joint fluid at revision TKA approached statistical significance, but represented only a small difference between the means. These data differ from previous reports, which typically showed a wide distribution of HA molecular weight as well as a wide spread in average molecular weights. Most previous reports[22,36,37] did not perform a proteolytic degradation, and did not dilute synovial fluid sufficiently to prevent non-Newtonian viscous effects from changing the results. This may have led to two problems: protein-HA interactions increasing the apparent HA molecular weight, and molecular weight distributions appearing much like the more concentrated injections (see Fig. 4.4.3). Dilution of synovial fluid samples in prior studies may have resulted in a more typical distribution of molecular weights, as found by Kvam *et al.*[26] The high correlation among measures of molecular weight (M_p, M_n, M_w, and M_z) speaks to the regularity of distribution of molecular weights. The present results suggested, then, that the distribution of molecular weight of HA in joint fluid is wide, but generally bell-shaped. The similarity of HA molecular weight distribution before and after TKA suggests that TKA does not necessarily degrade joint fluid enzymatically or mechanically.

4.6.5 Correlation between Hyaluronic Acid Concentration and Viscosity

The positive correlation between HA concentration and viscous parameters was expected, and has been noted previously.[38] The steady-shear viscosity of joint fluid greatly exceeds that predicted by the concentration of HA alone, however. Steady-shear viscosity of pure HA solutions has been reported in several publications.[39-43] None of these predict the flow properties of joint fluid reported in Chapter 3 given the HA content reported presently. As an example, two groups reported the rheological properties of HA (M_v 2.2 MDa[39] and 1.5 MDa[43]) at a physiological pH and variable ionic strength. The viscosity of these HA samples at 2 mg/ml were: 2.2 MDa, $\eta_0 = 0.05$ Pa s; 1.5 MDa, $\eta_0 = 0.02$ Pa s. Both are much less than the viscosity reported for synovial fluid presently (where $\eta_{1Pa} = 3$ Pa s at the same concentration and $M_w \sim 1.8$ MDa). This finding is

consistent with Swann's report of higher intrinsic viscosity from synovial fluid samples than would have been predicted by their HA molecular weight and concentration alone.[44]

The most likely source of these greatly increased viscous properties is interaction between proteins and HA. Interactions have been found between albumin and macromolecules other than HA through electrostatic, hydrogen, and hydrophobic interactions,[45] but HA is capable of each of these interactions as well. An interaction between proteins and HA has been suggested on numerous occasions[27,38] and is demonstrated by the necessity of proteolytic degradation prior to SEC in the present study. Furthermore, a number of studies have shown that the presence of proteins impacts the flow properties of joint fluid.[15,46]

A large portion of the wide variation in joint fluid flow properties is due to differences in HA concentration, as shown by the relatively high coefficient of determination for this connection. To support this finding, I refer to the work of Gibbs *et al.* In 1968, Gibbs *et al.* characterized the viscoelastic properties of HA (M_v = 2.2 MDa) in solution over a range of pH, concentration, ionic strength, and temperature.[39] They found an Arrhenius type correlation for temperatures from 3.5 to 25°C. They determined master curves for HA storage and loss modulus at physiological pH and ionic strength 0.0, 0.1, and 0.2. Flow properties varied unpredictably with ionic strength.

Steady shear viscosity at low shear rate, η_0, can be estimated from Gibbs' data using the relationship $\eta_0 = \lim_{f \to 0} G''/2\pi f$, where G'' is loss modulus and f is frequency of oscillation.[47] At low frequency, $G''/2\pi f$ could be estimated from Gibbs' charts to be 0.01 $a_C\, a_I$ (Pa s), where a_C and a_I are shift factors dependent on concentration and ionic strength, respectively. A summary of estimated η_0 for various ionic strengths and concentrations at pH 7 is given below in Table 4.6.1.

Table 4.6.1 Estimated parameters for viscosity of HA in solution Using the data of Gibbs *et al.*, this table estimates the ionic strength and concentration shift factors for HA at physiological pH (7.0), concentration (1 – 3 mg/ml), and ionic strength (0.15). From these, it estimates η_0. Calculations at 2 and 3 mg/ml are taken directly from Gibbs' data. Calculations at 1 mg/ml are extrapolated from data at 2 and 4 mg/ml assuming a geometric relationship. This means of estimation is intended to show the widest possible range given the available data. These data underscore the fact that HA concentration alone underestimates the flow properties of joint fluid.

Ionic Strength	*I = 0.1 (a_I =1.0)*			*I = 0.2 (a_I =5.2)*		
HA Conc.	*1 mg/ml*	*2 mg/ml*	*3 mg/ml*	*1 mg/ml*	*2 mg/ml*	*3 mg/ml*
$a_C\, a_I$	0.0077	1.0	35	0.99	5.2	17
η_0 (Pa s)	0.000077	0.010	0.35	0.0099	0.052	0.17

Examining Gibbs' results, it is clear that, given appropriate ionic strength, the flow properties of joint fluid (as estimated from η_0) could vary over more than three orders of magnitude based solely on a threefold change in HA concentration. Thus, the variation in flow properties could arise from differences in HA concentration alone. It should be noted that Gibbs' finding that ionic strength affects the importance of concentration in determining flow properties of HA has been disputed by others. Krause *et al.* argue that joint fluid ionic strength is in the high salt limit for HA, and therefore, there should be little effect of ionic strength on the flow properties of joint fluid.[43]

Finding that HA concentration correlated more strongly with d and η_{1Pa} than c and η_0, even when only samples fitting the Cross model were included, suggests that components of joint fluid other than HA most strongly affect flow properties at the lowest shear rates. This has been suggested implicitly by others,[27] and is consistent with the trend toward higher protein concentration and lower HA concentration found among samples which did not fit the Cross model. Rate index primarily depends on shear behavior at high shear rates, and η_{1Pa} is simply a measurement taken at relatively high shear rate in most joint fluid samples. Consistency and η_0, on the other hand, depend heavily on low-shear behavior, and were less well-correlated to HA concentration.

The absence of a correlation between η_{1Pa} and HA concentration among samples obtained at revision TKA is a difference between the index and revision groups. In this group, the lack of a correlation suggests a stronger contribution from the other components of joint fluid. One possibility is that the balance of proteins in TKA differs from that of the natural joint. The dominant proteins in TKA may have a greater effect on the flow properties of joint fluid than those in the natural joint do on synovial fluid. Alternatively, the influence of synovial membrane cells and articular chondrocytes in the natural joint may differ from their replacement joint counterparts, thus altering the relative importance of HA and other molecules in determining joint fluid properties. These hypotheses are consistent with the continued correlation between properties and phospholipids content in the absence of a correlation with HA content.

4.6.6 Correlation between Hyaluronic Acid Molecular Weight and Viscosity

The relatively low coefficients of determination for the correlation between HA concentration and viscous parameters imply that a significant amount of the variation in the data, even at high shear rates, cannot be explained by HA concentration alone. An obvious alternative source of this variability is HA molecular weight. No correlation between HA molecular weight and viscous properties was found upon first examination, but this was most likely due to the narrow range of average molecular weights found. When HA concentration was considered as a covariate, a correlation was found between HA molecular weight and viscous properties. The relationship was masked by the dependence on the highly variable parameter, HA concentration. An attempt was made to correlate concentration and molecular weight of HA to viscous parameters in one equation. Previously, η_0 has been shown to depend on the product of concentration (*conc*) and molecular weight (*MW*) in HA solutions.[48] Using this relationship, the correlation between HA and flow properties is strengthened ($p < 0.0001$, exponential regression $R^2 = 0.63$) for those cases fitting the Cross model (Fig. 4.6.1).

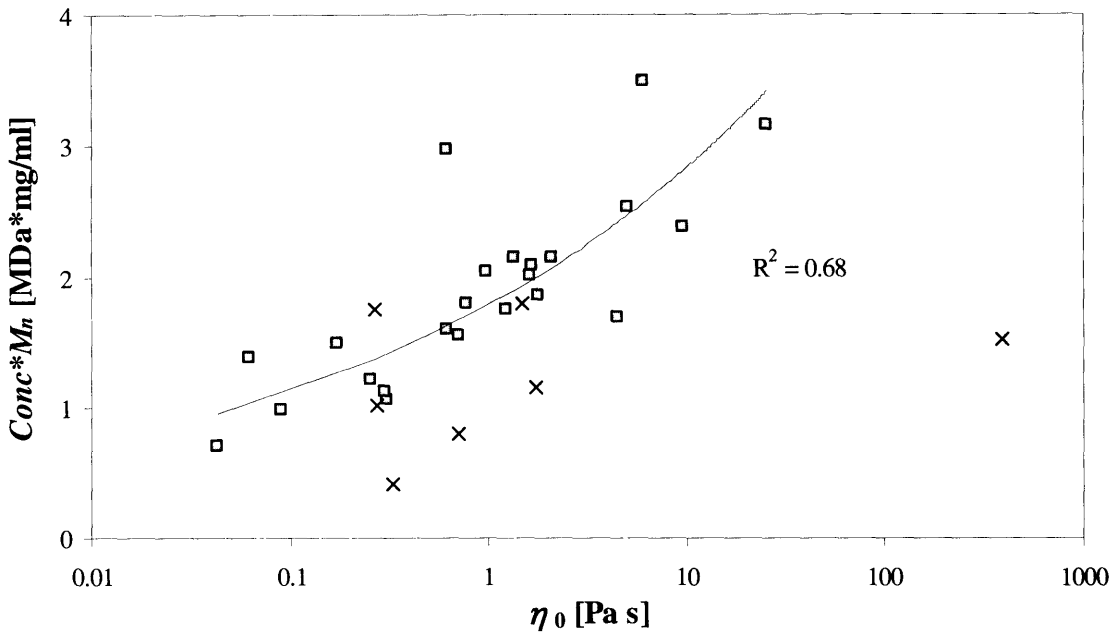

Figure 4.6.1 HA content versus low shear rate viscosity Gray squares represent samples that fit the Cross model, "x" represents samples that did not fit the Cross model. The ordinate is HA concentration times molecular weight. The thin line represents the best fit power law curve through the gray squares, with coefficient of determination shown. Exponential fit is discussed in the text.

A similar relationship has not previously been shown between HA parameters and *d*, however. In the case of polystyrenes, a relationship has been found between *d* and reduced concentration[49] (*i.e.*, concentration times intrinsic viscosity[50]). Assuming a power law relationship between intrinsic viscosity and molecular weight, as in the Mark-Honwink equation,[51] one can seek an analogous relationship in joint fluid between *d*, concentration, and molecular weight. A general form of this equation might take the form

$$d = A*conc*MW^{B}, \qquad \textit{Equation 4.6.1}$$

where *A* and *B* are constants. The data were fit to this model using a least squares fit to a power law relationship between the ratio of *d* to *conc* and *MW*, using each of the five measures of average molecular weight.

Both M_n and M_w strengthened the relationship between HA parameters and *d*, but neither M_z nor M_p did. For both molecular weight parameters, *B* was close to unity, consistent with the previous reports noted above. One would not expect this parameter to be rigidly defined by the present data, since the small variation in molecular weight limits its effect on viscosity. Nonetheless, more of the variability in rate index could be explained using both concentration and molecular weight of HA. Figure 4.6.2 shows a sample of the relationship between HA parameters and *d* in joint fluid. Even including both molecular weight and concentration, HA only accounts for 70% of the variation in flow properties of joint fluid, and does not explain the relatively elevated viscosity found, especially at low shear rates. The relationships shown in Figures 4.6.1 and 4.6.2 are consistent with the strong relationship between *d* and η_0 among samples fitting the Cross model, as discussed in Chapter 3.

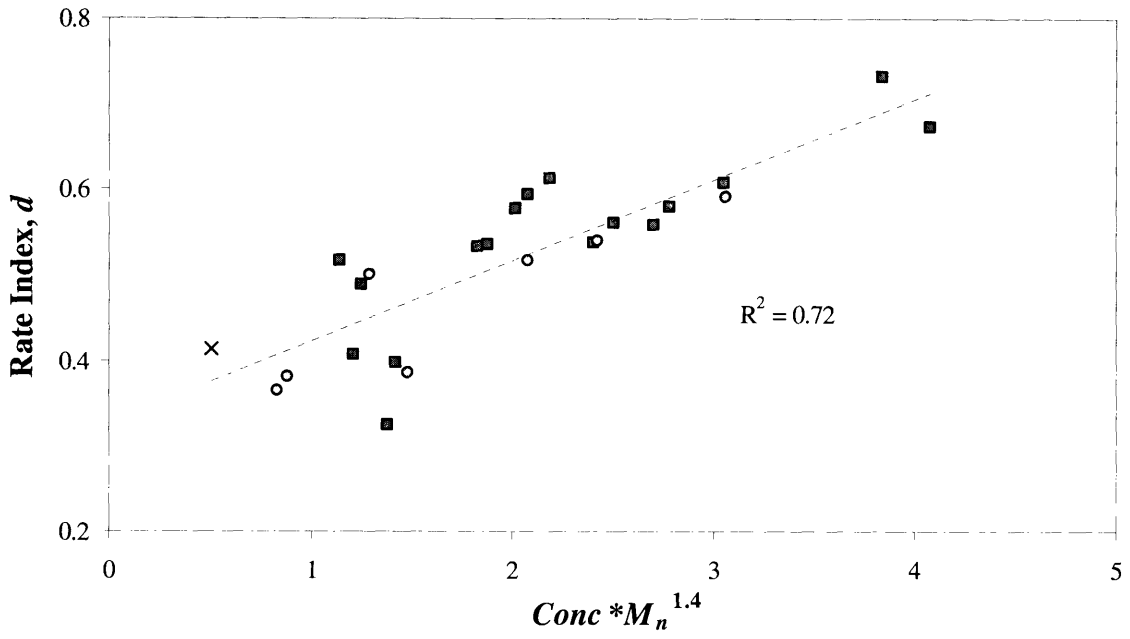

Figure 4.6.2 Rate index versus concentration times number average molecular weight to the power 1.4 Dark gray squares represent index TKA; light gray circles represent revision TKA, and the "x" represents the samples from an effusion subsequent to TKA. The units of the abscissa are left out for simplicity. The dotted line represents the best fit line through the data, with coefficient of determination shown. Better and more logical fits exist (*e.g.*, saturating exponential), but over this short range of data, a line is sufficient.

4.6.7 Effect of Other Components

Despite the above discussion, a substantial amount of the variability in joint fluid flow properties *cannot* be explained by HA concentration and molecular weight. The present data do not support the hypothesis that proteins-HA interactions explain the remaining variability in viscous properties of joint fluid. If this were so, a positive correlation would have been found between protein content and flow properties; the correlation was negative, however. Since there was also a negative correlation between protein concentration and HA concentration, the relationship between one pair of variables may have confounded the analysis of another relationship. When the relationship between *d* and HA concentration was accounted for, the effect of protein was removed, demonstrating that the additional variability was not caused by total protein concentration. The negative relationship between *d* and phospholipid was likewise eliminated when the relationship with HA concentration was considered.

Nonetheless, it may be that *particular* proteins present in small quantities interact with HA to substantially increase its viscous properties. Other components, including other proteins, may shield this interaction. The effects of such components would be missed by the present experiments. The relatively poorer relationship between HA and flow properties at low shear rate is consistent with protein-HA interactions affecting the viscosity, since protein-HA interactions have been shown to interfere with steady shear viscosity measurement in complex solutions, but only at low shear rates.[27]

There are other potential explanations for the remaining variability in results, however. For example, as shown above in Table 4.6.1, variable salt content may affect HA viscous properties,[39] though physiological variations within the high salt limit[43] are unlikely to have much impact. Alternatively, sugars have been shown to affect the properties of HA at low shear rate;[14,15] this may be relevant because glucose transport into the joint capsule may be affected by certain arthritic conditions.[52] Finally, since the behavior of the column above 2 MDa is not known, the molecular weight of HA could be underestimated, thus making the flow properties appear great relative to the HA content. HA solution at a concentration of 2.5 mg/ml would require an average molecular weight above 20 MDa to account for the flow properties of joint fluid, however. This molecular weight far exceeds any previous estimate of HA molecular weight in joint fluid. Thus, the limitations of the column are not likely the source of the unexplained variability in joint fluid flow properties.

Interestingly, Saari *et al.*, in their original work identifying the molecular weight of HA in synovial fluid, found almost a perfect correlation between "HA molecular weight" and viscosity.[22] As discussed in section 4.4, however, it is very likely that by not digesting protein prior to using SEC, this apparatus actually measured the effective molecular weight of an HA-protein complex. In fact, their correlation is likely nothing more than a correlation between two different means for measuring viscosity.

4.6.8 Correlation between HA and Viscoelastic Parameters

Viscoelastic moduli at all frequencies correlated better with HA concentration than viscous properties did. This positive correlation is consistent with the hypothesis that HA content significantly determines the elastic properties of joint fluid. The fact that viscoelastic moduli correlate more closely with HA concentration than viscous properties do is consistent with the finding that more molecular interactions affect viscous properties than affect elastic properties. Also consistent with this hypothesis is the relatively smaller disparity between joint fluid viscoelastic moduli and HA viscoelastic moduli given the same molecular weight and concentration.[14,15,41] The lack of a correlation between HA concentration and both f_c and G_c reflects the fact that relatively few samples exhibited crossover, preventing a meaningful correlation from being drawn.

4.6.9 Limitations of this Study

Important limitations of this study are similar to those reported in Chapter 3. Specifically, few samples were obtained from the same patients, and no samples were obtained from "successful" TKA. Future studies would benefit from evaluating samples from the same patients on a periodic basis, and from obtaining joint fluid from TJA patients at arthroplasty.

One limitation specific to this study was that the identity of the proteins in the joint fluid was not determined. Even though the total protein content was normal, the specific proteins present in these diseased joints may have differed from those present in healthy joints. The wide range of composition was reflected in the varied gross appearance of joint fluid found in this study and previously reported.[16] In particular, since fluid that appeared grossly normal had relatively high protein content, and fluid that appeared hemarthrotic or inflammatory contained "normal" amounts of total protein, it is

likely that the dominant proteins in these joints differ from those in healthy joints. Differing proteins in the joint may be due to altered filtration, or could result from differences in the endogenous production of proteins by the synovial cells. The effect of one particular protein may be different (or even in competition with) that of another protein. If the tribological impact of individual proteins in joint fluid were identified, it would be useful to know the concentration of each protein.

4.7 Conclusions and Relevance

The present experiments confirmed the primary hypothesis of this chapter: that the composition of joint fluid varies widely in TKA. Specifically, protein, phospholipid, and HA all varied widely, as evidenced by high standard deviations and large ranges of values. This finding is consistent with the hypothesis that joint fluid composition affects the tribology and therefore clinical outcome of TJA. The characterization of joint fluid from arthroplasty patients enables an analysis of the effect of physiological variation in joint fluid composition on the tribology of metal-on-PE articulation. This is the purpose of the following chapter.

Differences between primary and revision arthroplasty were suggested by the data, but were overwhelmed by the variability. Specifically, there was a trend towards higher protein content and lower HA content among samples obtained at revision due to wear-related failure. This finding was consistent with the general trends evident in various joint diseases, as reported previously by others. This result does suggest some differences between the OA synovial fluid and that present after arthroplasty.

In addition to the primary findings listed above, there were additional findings supported by the data. For example, protein and phospholipid were directly correlated among all samples, and both parameters were inversely correlated with HA concentration. These findings suggested a relationship between filtration and synthesis of joint fluid components by the synovial membrane. Since the synovial membrane largely determines the composition of joint fluid, an understanding of the synovial membrane in TJA is an essential prerequisite to the deliberate alteration of joint fluid in TJA. Furthermore, HA largely determined the flow properties of joint fluid, but neither the magnitude nor the variability of flow properties was explained by HA alone (especially at low shear rate, which is relevant for certain replacement joint articulations). This finding is useful in that it contributed to an understanding of the biochemistry of joint fluid, specifically with regard to the interaction between HA and other components of joint fluid.

4.8 References

This work is currently under consideration for publication in condensed form.

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CHAPTER 5

FRICTION AND JOINT FLUID

The effect of joint fluid on the tribology of TJA articulation has not yet been established, though its importance has been suggested. In the current study, a friction assay has been used to rapidly assess the effect of joint fluid and its principal components on the articulation of PE on Co-Cr. The course of experimentation to rank lubricants is validated by the relative performance of distilled water, saline, and bovine serum. Some joint fluid samples enabled very low friction similar to that of bovine serum, whereas others actually increased friction over that of saline. HA, albumin, and γ-globulin did not reduce friction relative to saline, but actually increased it, consistent with joint fluid samples that were poor lubricants. The lubricity of bovine serum was destroyed by proteolytic digestion. An alternate bearing surface, oxidized zirconium (Ox-Zr), showed reduced coefficient of friction relative to Co-Cr, and was not significantly affected by the composition of the lubricant. The findings suggest that water provides significant boundary lubrication of metal-PE bearings, but that a component of joint fluid, other than the components examined, provides additional lubrication for Co-Cr on PE. Further, the present study demonstrates that joint fluid is a patient factor that influences the tribology of metal-on-PE arthroplasty, and suggests a friction assay that could lead to clearer prediction of the likelihood of prosthesis failure initiated by the generation of PE wear particles *in vivo*.

5.1 Introduction and Objectives

The work of the previous two chapters demonstrates the considerable variability of joint fluid in TJA, but does not show that this variability impacts the tribology of TJA. The considerable variability in flow properties and composition of joint fluid in patients undergoing primary and revision arthroplasty is only relevant if this variation explains a portion of the unexplained variability in clinical outcome.[1,2] Consequently, a necessary step in this thesis is to examine the effects of the primary components of joint fluid (*i.e.*, water, proteins, phospholipids, and HA) on the lubrication of metal-on-PE. Since metal-on-PE TJA typically resides in a boundary or mixed regime, and since boundary lubrication relates to an interaction between surfaces and lubricants, it is appropriate to examine in more detail the boundary lubrication of TJA by the components of joint fluid evaluated in Chapter 4.

It has been shown that metal-on-PE replacement joints exist in a mixed or boundary lubrication regime[3-6] for many activities. As defined in section 2.1, boundary lubrication refers to the protective effect of particular lubricating molecules absorbed to a surface and repelling its opposing couple. Boundary lubrication is instrumental in the function of synovial joints. In this articulation, a protein specific to synovial fluid has been found to be essential for the very low friction between articular cartilage surfaces.[7-9] Consideration of the results of Chapter 4 in conjunction with the unexplained variability of wear rates in PE replacement joints suggests a connection between joint fluid composition and wear. Specifically, several components of joint fluid (such as water, protein, phospholipid, or HA) may perform some boundary lubricating function in the replacement joint, and variation in the content of any of these components could affect the tribology of TJA.

In this study, friction is measured in the articulation of a PE pin on a metal disk under a variety of circumstances corresponding to physiological conditions. These experiments are conducted for the purpose of identifying the contribution of each component of joint fluid to boundary lubrication in metal-on-PE.

5.1.1 Justification of the Use of a Friction Assay

Because of the morbidity associated with osteolysis linked to wear particles present in tissues surrounding joint implants, studies of the tribology of joint arthroplasty typically focus on wear volume and particle analysis. For many purposes, such as evaluating a new type of PE surface, this type of study is appropriate. The long time and high cost of joint simulator and pin-on-disk wear tests render them impractical as a means to screen a meaningful number of boundary lubricants, however. Furthermore, the volume of lubricant required prohibits direct tribological measurement of human joint fluid samples through wear experiments. Consequently, in the present study, a friction assay under boundary lubricating conditions was developed to rank lubricants.

In contrast to wear experiments, the friction assay used presently requires only about 3 ml of lubricant per test, and can be conducted in a few minutes. The low expense and time required enable more testing iterations. Another advantage of the friction apparatus is its relative simplicity, which makes data more repeatable and interpretation of data simpler than from typical wear experiments in the arthroplasty field. It is believed

that these advantages outweigh the disadvantages of using friction apparatus; namely, that the clinical problem is the generation of wear particles, rather than the generation of friction.

There is intuitive basis for the use of a friction as a tribological assay. Frictional force represents the amount of work generated in articulation per unit distance slid. For metal articulating on PE, this work is dissipated in processes such as heating, plowing, and deforming the PE surface. Since these interactions lead to adhesive wear, abrasive wear, and gross damage, differences in friction in short times indicate differences in wear over long times. Thus, for a given wear mechanism and wear surface, higher friction suggests more wear particle generation. Therefore, one can compare the relative tribology of two lubricants in a metal-PE couple by comparing their lubricity; that is, by comparing the friction generated by the articulation when lubricated by the two different fluids. Furthermore, one can compare the relative tribology of two counterfaces on a single PE surface if the PE surface is identical and the lubricant used is physiologically appropriate. This intuitive argument is supported by a mechanistic explanation of friction and wear in metal-on-PE articulation (see sections 5.9 and 6.5).

Additional support for the use of a friction assay comes from its use historically. Friction assays have been used in cartilage on cartilage systems[10,11] and latex on glass systems[7,11-13] to demonstrate the presence of a boundary lubricant for synovial joints in healthy synovial fluid. In recent years, a number of other researchers have used friction measurements to determine tribological differences among lubricants in replacement joint couples as well.[14-16] These tests have employed friction because friction is believed to be a reliable assay for the performance of boundary lubricants.

A final source of support for this type of assay comes from empirical data. In a recent hip simulator study, Wang *et al.* measured friction and wear in the articulation of PE on Co-Cr, and reported a strong correlation between the two.[17] A similar relationship was also shown in a knee simulator, in which increased friction was associated with increased wear.[18] Moreover, POF experiments described in Chapter 6 show a similar relationship between friction and wear. Finally, in a finite element model, Teoh *et al.* found wear to increase with friction in THA.[19] Thus, in addition to an intuitive argument for a relationship between friction and wear under the conditions of these tests, historical use of a friction assay and empirical findings support its use to identify a boundary lubricant for metal-on-PE in joint fluid.

5.1.2 The Importance of a Physiological Lubricant

It is necessary to determine a physiologically relevant lubricant for use in laboratory studies because the tribology of the surfaces may be different under different lubricants. For example, in recent tribological tests, a ceramic-on-ceramic articulation performed much better than metal-on-metal with a silicone-based lubricant, but using joint fluid, the metal-on-metal articulation performed better.[5,6] Again, although, metal-on-PE articulations have traditionally performed better under serum lubrication than water, PE-quartz composites have shown the opposite lubricant ranking.[20] Thus, tribological behavior under a particular lubricant and material couple does not necessarily predict tribological behavior under another lubricant and material couple. McKellop stated this conundrum well in 1978: "It might be argued that even though wear in distilled water is qualitatively different than that in serum, the results of distilled water

tests might be useful in establishing the relative wear rates of various combinations of materials. This does not appear to be a safe assumption. In addition to the marked differences in wear properties demonstrated in this study with polyethylene, other materials have been shown to have wear rates strongly dependent on the lubricant used."[1] Ironically, though he used this argument to favor bovine serum over distilled water, this same argument shows that bovine serum is unsatisfactory for the purpose of simulating joint fluid in TJA tribology experiments. Instead, it is necessary to determine which components of joint fluid contribute to tribology of TJA and how they do so, so that these may be used in laboratory studies. This argument also underscores the need to understand more clearly the effect of joint fluid on the tribology of TJA to enable proper experimental design and interpretation of laboratory studies.

5.1.3 Specific Aims and Hypotheses

There were several aims of this Chapter. Before testing any hypotheses relating to the effect of lubricants on friction in metal-on-PE articulations, it was necessary to demonstrate that a friction assay can distinguish among lubricants. Specifically, an appropriate friction assay would demonstrate that PE on Co-Cr has a lower coefficient of friction when lubricated by bovine serum than when lubricated by distilled water or saline. It has been established that metal-on-PE generates several-fold less wear when lubricated by bovine serum than when lubricated by water (cf. section 2.4.6). A friction assay that can identify the difference in tribology between bovine serum and water is likely to identify other differences of similar magnitude.

Once the validity of the experimental apparatus for this purpose was established, the apparatus was used to achieve several specific objectives. Friction was measured between Co-Cr and PE lubricated by a number of different joint fluid samples. It was hypothesized that different samples would bring about a range of friction values, varying from that of water lubrication to that of serum lubrication. This hypothesis, when considered in conjunction with the demonstration in Chapters 3 and 4 that joint fluid varies in arthroplasty, is the crux of the thesis. If verified, it would demonstrate that joint fluid is a primary determinant of tribology in TJA. Second, experiments were conducted to determine the contribution of each of the primary components of joint fluid (albumin and γ-globulin, phospholipid, and HA) to boundary lubrication in TJA, both in PE on Co-Cr and PE on Ox-Zr. Specifically, it was hypothesized that the friction between Co-Cr and PE could be predicted by the protein, phospholipid, and HA content alone. A related aim was to examine the relative tribological impact of replacing Co-Cr with Ox-Zr as a counterface for PE. Third, a pilot study tested the relative importance of proteins not previously examined by examining the effect of protein digestion on the lubricating ability of bovine serum in PE on Co-Cr articulation.

The final aim of this study was to bring together an understanding of these results within the model of a better conceptual understanding of boundary lubrication of metal-on-PE TJA.

5.2 Materials

5.2.1 Friction Apparatus

The apparatus used to measure friction was a simple device essentially the same as other devices that have been used to measure friction between articulating couples in other fields (*e.g.*, Komvopoulos *et al.*[21]). The custom apparatus (Fig. 5.2.1) consisted of a cantilever arm and turntable. The PE pin was fixed to one end of the cantilever, onto which a dead weight was applied, providing the normal force. The other end of the cantilever arm was held fixed. The tip of the pin rested on a metal disk fixed to the turntable. Lubricant was dispensed onto the disk along the circumferential path of the pin while the pin was kept apart from the surface, thus ensuring complete exposure of the frictional interface to lubricant. When the pin was released, a load applied, and the disk-turntable assembly rotated, the frictional force was manifest in a transverse force applied to the pin and cantilever arm. The resultant transverse displacement of the cantilever was measured by strain gauges, which recorded the displacement on computer via a voltage output. This voltage could then be converted to a frictional force through a separate calibration performed regularly.

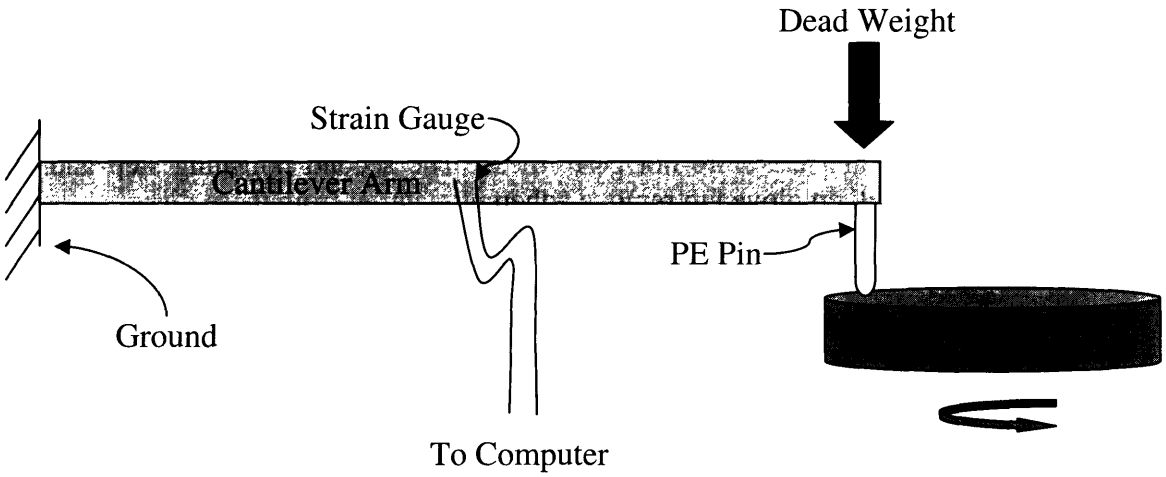

Figure 5.2.1 Schematic of apparatus to measure friction For clarity, the lubricant bath and the motor controlling disk rotation are not included in this drawing. This simple turntable-style apparatus is similar to many used to measure friction between various couples.

5.2.2 Polyethylene Pins

The cylindrical PE pins were provided by Smith & Nephew (Memphis, TN), and machined from accepted rod stock of PE (GUR 1150; Westlake Plastics, Lenni, PA). One pin was 6.4 mm in diameter, with flat ends machined to implant grade smoothness. The remaining pins were 3 mm in diameter, having the articulating end machined into a spherical shape of 3, 4.5, 6, or 9 mm diameter. All pins were 20 mm in length. The pins were used as received and were not sterilized. After the tests, pins were examined both grossly and microscopically (SZ-PT optical microscope, Olympus, Japan).

5.2.3 Metal Disks

The metal counterfaces were flat disks 50 mm in diameter and at least 6.5 mm in thickness, made of Ox-Zr or accepted bar stock Co-Cr polished to implant grade specifications; both were provided by Smith & Nephew (Memphis, TN). Between tests, disks were polished to a mirror finish manually using a multi-step process. First, disks were rinsed of contaminants in tap water followed by distilled water. Then, disks were polished for one minute using 0.3 μm Alpha Alumina Micropolish solution (Buehler, Lake Bluff, IL) on an 8 inch diameter micro-cloth (catalog number 40-7218, Buehler, Lake Bluff, IL) or equivalent polishing surface fixed to a Rotopol-1 (Struers, Copenhagen, Denmark) operating at 150 rotations per minute. Then the disks were rinsed again in tap water followed by distilled water. In the second polishing step, disks were polished for a second minute by Mastermet Colloidal Silica Polishing Suspension (catalog number 40-6370-064, Buehler, Lake Bluff, IL) on an 8 inch diameter micro-cloth (catalog number 40-7218, Buehler, Lake Bluff, IL) polishing surface fixed to a Vari/Pol VP-50 (Leco Corporation, St. Joseph, MI) operating at 150 rotations per minute. The disks were soaked in distilled water for 30 seconds, then rinsed thoroughly in distilled water, and finally dried by forced warm air convection using a hand dryer. The disks underwent a final cleaning step by ultrasonication for ten minutes in distilled water before being used again in further experiments. When scratches or contaminants remained on the disk surface after any step in the process, the most recent polishing step was repeated. Because scratches did not appear on the Ox-Zr disks, the first polishing step was superfluous and therefore not performed. The second polishing step and all cleaning steps were still employed to remove adherent material from these disks.

5.3 Pilot Comparison of Co-Cr to Ox-Zr

A pilot study investigated modes of lubrication in the articulation of replacement joint materials under physiological conditions using the friction apparatus of Fig. 5.2.1. It was hypothesized that both boundary and mixed lubrication modes could be shown in the articulation of replacement joint materials within a physiological range of stress and velocity. A secondary aim of this study was to compare two different metal surfaces currently in clinical use for articulation on PE in this application. This study was intended to lay the groundwork for future investigations into the boundary lubricating ability of various components of joint fluid.

Co-Cr on PE was the obvious choice as one articulating couple because it is the most commonly used replacement joint couple. The second pair, Ox-Zr on PE, was chosen because it is a newly-approved couple for use in replacement joints. Ox-Zr is a zirconium alloy (2.5% niobium) whose surface has been passively oxidized to create a ceramic surface. The manufacturer of Ox-Zr has claimed that the material generates a lower coefficient of friction than Co-Cr when articulated on PE.

5.3.1 Additional Materials and Methods

The top articulating surfaces, made from implant stock PE, consisted of 20 mm long cylinders of diameter 3 mm. One end of each pin was flat, and the other had a spherical tip of 3, 4.5, 6, or 9 mm diameter. A convex pin simulated the geometry of replacement joint articulation more closely than flat-on-flat articulation. Furthermore, the

use of a hemispherical pin simplified the calculation of contact stress and contact area. The range of diameters was intended to provide a large, physiological range of Hertzian contact stresses with small variation (60 to 300 g) in normal load.

This initial study was conducted before an adequate calibration protocol had been developed. Consequently, only qualitative results are discussed, and no figures are given. In these initial studies, the two metal types were compared as counterfaces to PE under serum lubrication conditions. These surfaces were tested under 6 different loads. A dead weight with mass 62.8, 82.2, 127, 139, 215, or 299 g was placed on the pin to provide a controlled normal load. Under each load, measurements were taken each second for at least thirty seconds under clockwise and counterclockwise rotation at three velocities: 0.01, 0.02, and 0.04 m/s. The speeds and load were chosen so as to represent physiological conditions. Furthermore, the loads and speeds were varied to identify the dominant modes of lubrication. The strain gauges were calibrated using known transverse forces. Between experiments, the lubricant was discarded, and the pin and disk both rinsed thoroughly.

5.3.2 Hertzian Contact Stress Analysis

Real area of contact and average contact stresses were calculated using Hertzian analysis.[22] A sample calculation for 9 mm diameter pin under 299 g normal load is given here.

Since one body is a sphere and the other is flat, the reduced radius, R', is half the radius of the sphere, or 2.25 mm. Given that the metal surface is much stiffer than the PE surface, and assuming a reasonable Poisson's ratio (υ_{PE}) and Young's modulus (E_{PE}), the reduced modulus can be estimated as

$$E' = 2E_{PE}/\left(1-\upsilon_{PE}^2\right) = 2\times 1.0/\left(1-0.4^2\right) = 2.4 \text{ GPa.} \qquad \textit{Equation 5.3.1}$$

The contact area, A, is then given by πa^2, where $a = \left(3WR'/E'\right)^{1/3}$ = 0.20 mm and W is normal load. Thus, the contact area is 0.13 mm^2, and the average contact pressure, which is load divided by area, equals 22.7 MPa. Similar treatment using different values for R and W yield the range of values (13 MPa to 47 MPa) used in these tests.

Most of the assumptions of Hertzian contact stress[23] are valid in this articulation. The contact can likely be described by a continuous polynomial. The surfaces are isotropic and exist at quasi-equilibrium. There is no stress far from the zone of contact and no normal stress outside the zone of contact. Contact would drop to a point if no load were applied, and the integral of the normal stress equates to the normal load. Although the surfaces are not precisely smooth, the metal is polished sufficiently that the deformed PE articulates on a smooth surface. The two assumptions not quite met are that the distance between bodies is zero (lubricant may generate a small gap on the order of tens of nanometers) and that the tangential stress is zero (rotation generates a frictional tangential force). Despite these last two conditions, it is believed that the average contact pressure can be estimated using Hertzian analysis.

5.3.3 Choice of PE Pin and Metal Disk

The geometry that would best match clinical conditions is a metal pin on a PE disk, since the convex surfaces in TJA (femoral heads and femoral condyles) are usually

metal, and never polymeric. The specified pins could not have been manufactured using Ox-Zr, however, so this geometry could not be achieved. In particular, the oxidization process used to coat the surface of Ox-Zr does not allow for the diametric specifications required. The calculated contact stresses would have been subject to too much error, obfuscating true friction measurements. Consequently, PE pins were chosen to articulate on metal disks. A benefit of this choice was that the PE pin deformed only once, before the start of articulation. A metal pin would continually deform a new PE contact area, thus introducing a viscoelastic effect of PE deformation.[24,25] Using a PE pin rather than a metal one prevents this additional confounding parameter.

The 3 mm pin diameter was chosen based upon the relatively small disk size. By varying the diameter of the spherical tip, it was possible to obtain average contact stresses in the range 13 MPa to 47 MPa. These values are at the high end of the range of contact stresses found in replacement hips by finite element modeling[26] and pressure-sensitive film.[27,28] However, both these methods have limitations, and may underestimate the true contact stress in TJA, as discussed in section 2.4.2. Furthermore, extreme conditions can identify a good lubricant in a rapid assay, whereas, under physiological conditions, tribological differences may become evident only after extended tribological evaluation.

5.3.4 Pilot Study Results and Discussion

In all experiments, steady-state dynamic frictional force was achieved within two seconds. The first data point at a given velocity and direction was removed, and the remaining 30 or more provided the mean result, with standard deviation on the order of 10% of the mean.

Considering all thirty data points generated during one pin-on-disk experiment, and assuming a coefficient of variation of 0.10, differences of 7% between mean values are statistically significant by Student's *t*-test ($p < 0.05$).[29] This analysis was not conducted for all groups because the averaging of 30 data points from one experiment is not truly appropriate to determine significance. Multiple experiments must be performed using multiple pins or disks. Nonetheless, the rudimentary statistical analysis above suggests that multiple tests will bring about statistically significant differences between groups. For this pilot study, it was sufficient to analyze the data assuming statistically significant differences.

At low loads, there was an apparent relationship between coefficient of friction and load, with coefficient of friction increasing as load decreased. At higher loads, this relationship no longer applied. Given the choice of geometry, there was no chance of fluid film lubrication between these surfaces. Therefore, the explanation of a transition from boundary to fluid film lubrication was not appropriate. The relationship between load and coefficient of friction could be well described by a power law throughout the range studied. Further consideration of this relationship in metal-on-PE is discussed later in this chapter. As evidence of boundary or mixed lubrication, coefficient was largely independent of velocity throughout these pilot tests.

Finally, a direct comparison was made between Co-Cr and Ox-Zr. At all pin sizes except 6 mm, Ox-Zr exhibited a slightly lower coefficient of friction than Co-Cr. In this pilot study, there were not yet enough data to make a meaningful statistical comparison between the two surfaces, but the initial data suggested that Ox-Zr has a slightly lower

coefficient of friction than Co-Cr. If a difference between Co-Cr and Ox-Zr could be found, that difference might have to do with the wettability of the surfaces.

For this pilot study, bovine serum was chosen as a lubricant to conform to ASTM standard F732-00.[30] Although this standard guides wear tests, rather than friction tests, it seemed an appropriate baseline for comparison. Furthermore, the Ox-Zr manufacturer's claim of a lower coefficient of friction compared with Co-Cr was based upon experiments lubricated in bovine serum.

5.3.5 Pilot Study Conclusions and Limitations

From this study, it appeared that this experimental apparatus may be useful for examining the tribology of TJA, but that additional data were necessary before conclusions can be drawn. There were four major limitations of this study. First, although each piece of data represented an average of thirty or more data points, the inter-experimental variability of tribological experiments made it more appropriate to consider each experiment as a single data point. Thus, several experiments must be run in order to attach error bars to the data. Consequently, data analysis was limited by the shortage of data points.

Second, velocity in these experiments was not easy to control, though it was accurately measurable. The motor providing rotation was torque-controlled, and therefore resulted in a variable velocity depending on the frictional resistance. Furthermore, motion in one direction contained more internal motor resistance than motion in the other direction, so the two directions did not necessarily provide the same velocity. Consequently, it was necessary to adjust the continuously variable torque controller to produce comparable velocities under different conditions. In future work, friction is determined only in one direction, to remove any disparity between the two directions of rotation. For each experiment, the time required to make one or more revolutions was used to calculate velocity. The different velocities are not discussed above. In the statistical analyses, the actual velocity (not the intended velocity) was considered. Because friction was largely independent of velocity, remaining work employs only one velocity, 20 mm/s.

Third, the geometry used did not match the geometry of the joint. This may seem a self-evident and trivial point, but there may be important ramifications of using a PE pin and metal disk instead of vice versa, which would better simulate the geometry of the joint (convex metal part, concave PE part). As an example of the significance of the difference, consider the cyclical compression of PE. Since a PE pin is continually under compressive load, it undergoes a constant deformation, whereas a point on a PE disk would be under compression only for a small portion of a cycle. Thus, it would undergo a cyclical compression, rather than a continuous one. There may be frictional losses associated with this cyclical motion. Nonetheless, as long as comparisons are made between identical geometric designs, these comparisons can be meaningful.

Finally, bovine serum is very different from joint fluid, and may lubricate quite differently. A material combination that performs very well in one may be weak in the other. Future tests employ fluids more closely representing the relevant components of joint fluid.

5.4 Effect of Load and Pin Size

The pilot study suggested that Ox-Zr would bring about reduced friction relative to Co-Cr. To validate the experimental apparatus, and to determine which conditions were best suited to measuring tribological differences, the relationship between normal load and frictional force was determined for PE on Co-Cr using distilled water, serum, and two different joint fluid samples as lubricants. The frictional force was determined under loads of 59.9, 299, and 589 g, using a 6.4 mm diameter flat pin and a 3 mm diameter pin with 3 mm diameter spherical tip. Specifically, it is generally accepted that water lubrication brings about higher wear rates and higher variability in wear rates than bovine serum lubrication for the articulation of PE on Co-Cr. Frictional differences between these two lubricants would demonstrate the utility of the apparatus.

5.4.1 Additional Materials and Methods

Four lubricants were employed in this study. Newborn bovine calf serum (catalog number 12133-78P, lot number 002006A; Invitrogen Corporation, Grand Island, NY) was diluted to 40% v/v in distilled water and compared to 100% distilled, deionized water as control groups. Additionally, one joint fluid sample was obtained from an OA patient TKA (Study ID 018) and one sample was obtained from a TKA patient at revision surgery (Study ID 201). The second sample was obtained after the studies of Chapters 3 and 4 were completed, so it is not recorded in Appendices A and F. Its properties and composition have not been measured. In this case, revision was performed eight years after primary TKA because of PE wear and osteolysis. Each lubricant was tested six times, except for the OA case. In this case, there was only sufficient fluid for two experiments.

Experimental Protocol

Each lubricant was tested under three different loads (59.9 g, 299 g, and 589 g) using two different types of PE pins (6.4 mm flat and 3 mm spherical tip) as described in section 5.2. After 1 to 3 ml of lubricant had been applied to the metal counterface, the PE pin was brought in contact with the lubricated metal and loaded. The disk was then rotated in reverse for at least one revolution, to standardize any preload or initial offset in the cantilever arm. Upon initialization of forward rotation, maximum static friction (μ_s) was recorded as the largest positive output within 0.25 seconds, with measurements taken at 40 ms intervals. Then, after the disk rotated for 30 seconds to reach steady state, mean dynamic friction (μ_d) was measured using the mean output over the next 40 seconds, with a one second sampling interval. The mean dynamic friction was considered a single data point even though it was determined by averaging 40 points in 40 seconds. Measurement in only one direction of rotation reduced the error introduced by differences in motor and cantilever behavior in opposite directions, and thus necessitated calibration only in one direction. All experiments were run at a velocity of 20 mm/s, though preliminary experiments were performed at 10 and 40 mm/s to demonstrate that the friction measured was independent of velocity.

When the experiment was completed, as much lubricant as possible was removed from the disk, and the disk was removed from the cup and evaluated visually, specifically looking for scratches and evidence of PE transfer. A new disk was placed in the lubricant

cup, and the lubricant was pipetted back onto the surface. When necessary, additional lubricant was added to replace that which could not easily be removed from the previous disk.

Calibration

To calibrate the device, a string and pulley were attached to the end of the cantilever arm, enabling free weights to apply a force to the cantilever in the direction of friction. Masses from 1.01 to 7.03 g were used to determine a relationship between applied force and voltage output. The baseline voltage was adjusted so that the output was zero volts when no force was applied. A proportional relationship was confirmed between the voltage output and applied force. At first, this calibration was performed at the start of each experimental session (daily). It became quickly evident that this was unnecessary, however. The calibration was thus performed monthly to confirm that the properties of the cantilever system had not changed. See Appendix M for a more detailed discussion of the calibration process.

5.4.2 Results

Effect of Test on Pins and Disks

Upon gross and microscopic examination, the PE pins were not affected by the tests despite the fact that applied stresses exceeded the yield strength of PE. In preliminary tests, use of the pins for more than twelve tests (more than two test groups) resulted in microscopically visible damage to the PE surface. Consequently, in all the tests related to the present experiments, pins were used only for six or twelve consecutive experiments (one or two test groups).

On occasions when lubricants other than distilled water or saline were used, components of the lubricant visibly adhered to the metal surface. Occasionally, a scratch could be seen on a Co-Cr surface upon completion of a test. Finally, in many cases, there was evidence of a transfer film between the PE and metal surfaces. This evidence typically consisted of a dry track with sharp borders to a wetted surface, consistent with the path traversed by the pin rendered hydrophobic. This evidence of transfer film was not associated with increased coefficient of friction, and typically, no damage or material loss from the PE surface could be observed grossly or microscopically.

These results characterized the effect of the tests on pins and disks throughout the experiments of this chapter. This discussion is therefore not repeated in each section.

Typical Output Curve

The general behavior of friction under this protocol (used for all subsequent friction tests) was as follows (Fig. 5.4.1). Prior to initialization of motion, friction was typically close to but less than zero due to reverse rotation prior to the test. At initialization of motion, friction increased rapidly over the first hundred milliseconds, and then oscillated about a relatively high value. Occasionally, the peak value during these oscillations exceeded the static friction value measured within 0.25 seconds of initialization of motion (not the case in the sample shown in Fig. 5.4.1). In all cases, the amplitude of oscillation decreased during the first 30 seconds of motion, as did the

magnitude of friction. During this time, friction was not recorded. After 30 seconds, irregular variation in friction continued, sometimes with peak-to-valley differences of the same order of magnitude as the mean friction value. No further reduction in friction variation was evident after 40 seconds of continuous measurement (70 seconds of sliding).

The form of the results was consistent with smaller scale polymer articulation measurements, which reported static friction, oscillation, and a final value in the midst of continued oscillation.[31] Using our apparatus, it appeared that the decay of oscillation was much slower than that in smaller scale experiments. This variation (standard deviation of 3.0 g in Fig. 5.4.1) was much greater than the resting noise in strain gauge measurement (± 0.6 g), so was not likely due to imprecision inherent in the strain gauges. The period of these regularly irregular oscillations at steady state was consistent with the period of rotation of the metal disk. Based upon the similar periodicity of output oscillations at startup and steady-state using different sampling intervals, it is believed that the oscillatory output represents time-variation in F_f, rather than signal processing noise. The fact that the period appeared to be equivalent to the period of disk rotation was consistent with the expectation that interactions between the PE pin and metal asperities would generate friction between the surfaces.

Using the typical output curve given in Fig. 5.4.1 as a guide, differences of 1.8 g between two measurements of dynamic friction are statistically significant ($\alpha = 0.05$, $\beta = 0.05$). Since this represents a miniscule difference in μ_d, this parameter was determined from the output curve as described in section 5.4.1. The standard deviation bars obtained from a single test were not used in the analysis of the data.

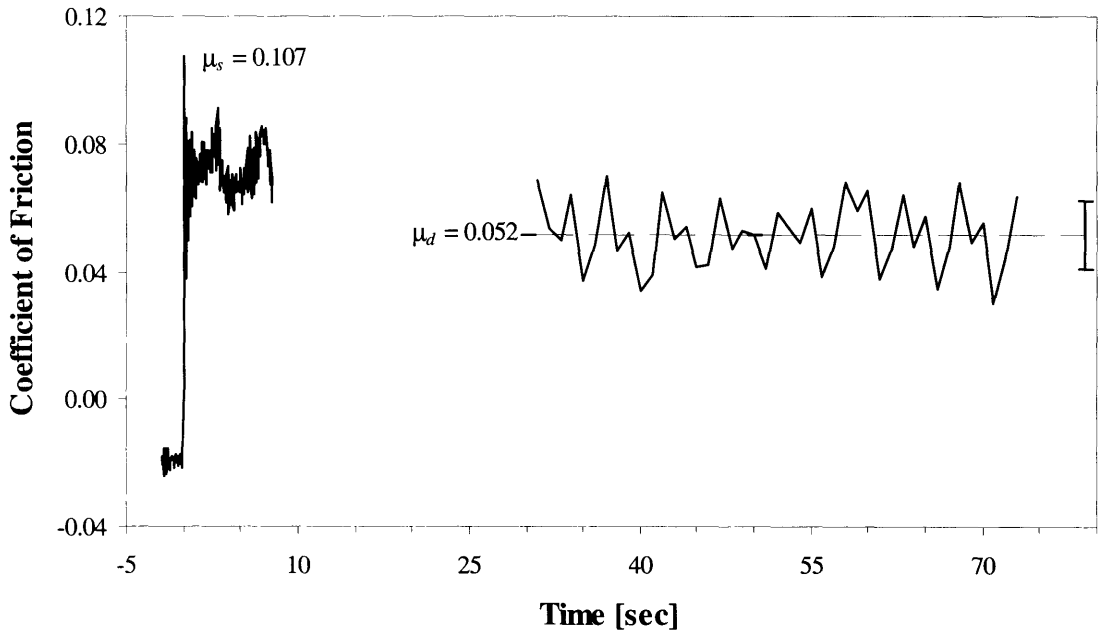

Fig. 5.4.1 Friction output versus time from a typical friction test The force output versus time of one case of a joint fluid sample (Study ID H01, disk 2) is given. Voltage output has been converted to force output, and plotted as coefficient of friction for this chart. The initial output is shown at initialization of motion (time = 0). Note the rapid sampling interval for the initial portion of the test, with μ_s given. The dotted line indicates μ_d, with error bars representing standard deviation at the far right.

Results of Load and Pin Size

Under low load (59.9 g), for all lubricants and both pin types μ_d was close to 0.1 and μ_s was close to 0.3 (Figs. 5.4.2 & 5.4.3). Friction was higher for the spherical pin than for the flat one. As load was increased to 299 g, frictional force increased as well, but the coefficient of friction decreased, both for spherical and flat pins. This trend continued at the highest (589 g) load. At the highest loads, friction was lower using the spherical pin than the flat one. Coefficient of friction decreased more under serum lubrication than under water lubrication (μ_s, $p = 0.0044$, μ_d, $p = 0.0007$). Of the two joint fluid samples evaluated under both high and low loads, one enabled friction less than that of serum, though the difference was not statistically significant, and one had friction more than that of water (μ_d, $p < 0.0001$). For both a flat and spherical pin, serum and one joint fluid sample exhibited low friction, water exhibited higher friction, and the other joint fluid sample still higher friction. The differences among lubricants were larger and more significant using spherical pins than they were using flat pins, and under highest load than under intermediate load. Consequently, 3 mm spherical pins and 589 g loads were used exclusively for the remainder of the experiments.

Based upon an assumed PE elastic modulus of 1000 MPa[32,33] and a Poisson's ratio of 0.4, the average Hertzian contact stress under this load was 59.2 MPa.[22] Although these loads exceed those typically estimated in hip and knee arthroplasty by finite element models[26] and pressure sensitive film,[28] it is recognized that these tools likely underestimate the actual stresses applied to PE in arthroplasty. Furthermore, since large differences in wear over the long term may be indicated by small changes in friction, relatively extreme conditions may be necessary to elucidate tribological differences in a short test. Loads higher than 589 g were not employed because the displacement corresponding to 111 g tangential load saturated the means of data acquisition, so forces above this value were truncated. At 589 g normal load, the maximum force measured corresponded to $\mu = 0.19$, which was rarely achieved in static friction and never reached in dynamic friction. Using flat pins, two of six experiments under serum lubrication and one of six tests under water lubrication exceeded this maximum in static friction under highest load.

Under the highest load, there were statistically significant differences among the samples. The TKA case brought about higher friction than the OA case (μ_s and μ_d, $p < 0.0001$), bovine serum (μ_s and μ_d, $p < 0.0001$), and distilled water (μ_s, $p = 0.005$, μ_d, $p = 0.024$). The OA case brought about lower friction than water did (μ_s, $p = 0.011$, μ_d, $p = 0.004$). Bovine serum was not significantly different from the OA case. Comparing the 3 mm spherical tipped pin to the flat pin, friction was lower for the 3 mm pin (μ_s, $p = 0.05$, μ_d, $p = 0.0005$). Importantly, bovine serum brought about lower friction than distilled water. Using both flat and spherical pin data, the difference was statistically significant for dynamic friction ($p = 0.01$) but not for static friction ($p = 0.057$). The difference between water and bovine serum is more highly significant when the spherical pin only is used. This is discussed further in later comparisons. Nonetheless, the difference between bovine serum and distilled water is essential to the demonstration that this apparatus distinguishes among lubricants. In particular, we find that a small but significant reduction in friction is associated with a large difference in PE wear rate. This finding is used as the basis of further work using this apparatus.

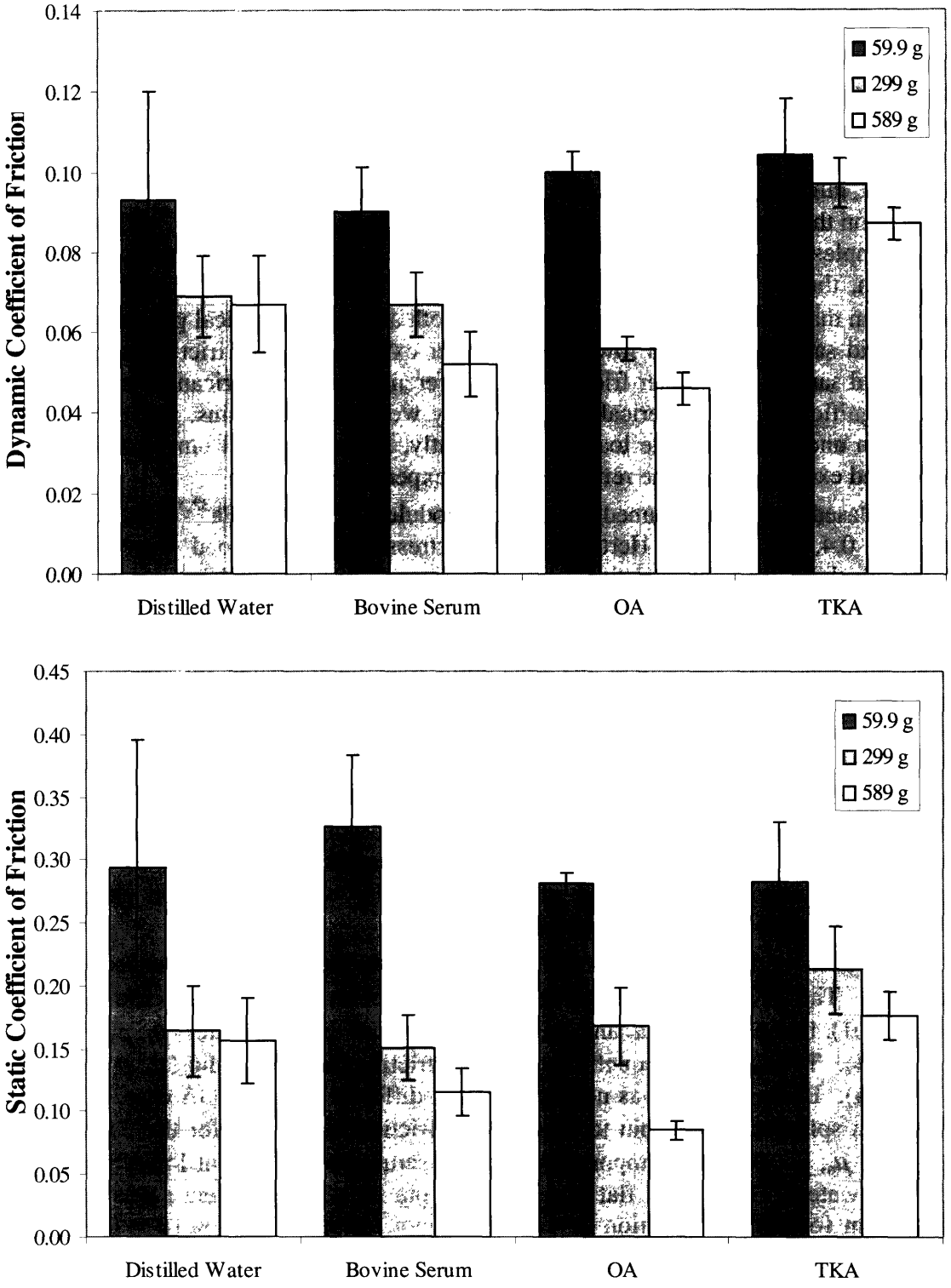

Fig. 5.4.2 Static (below) and dynamic (above) friction for a 3 mm diameter spherical-tipped PE pin on Co-Cr under a variety of loads and lubricants Dark columns represent low load, light gray columns represent intermediate load, and light columns represent highest load. Bars represent standard deviation. Dynamic friction of the TKA case was significantly greater than that of all the others ($p < 0.0001$).

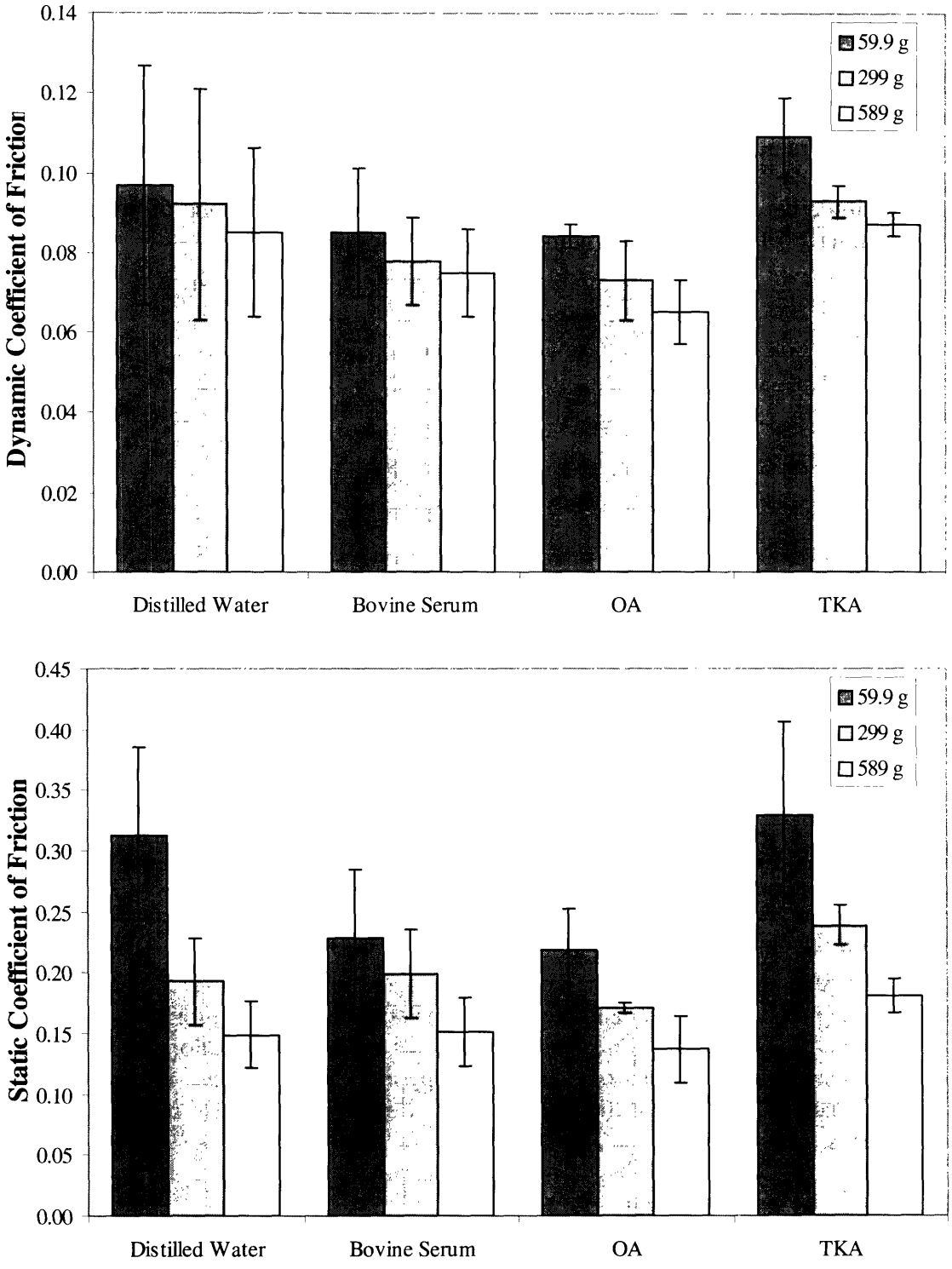

Fig. 5.4.3 Static (below) and dynamic (above) friction for a 6.4 mm diameter cylindrical PE pin on Co-Cr under a variety of loads and lubricants Dark columns represent low load, light gray columns represent intermediate load, and light columns represent highest load. Bars represent standard deviation. Dynamic friction of bovine serum and the OA case are significantly lower than that of the TKA and water cases ($p < 0.04$). A similar trend is seen in static friction, though differences were not always statistically significant.

5.4.3 Discussion

Previously, a power law relationship had been reported for metal-on-PE friction under dry and lubricated conditions.[34] Although three data points are not sufficient to *demonstrate* a power law fit (even when load is varied over an order of magnitude), the present data are consistent with the use of a power law model to relate friction to normal load (Fig. 5.4.4). The exponent in this power law relationship for the four different fluids is found to be between 0.66 and 0.93, consistent with previous reports of this power law relationship.[34-36] In Fig. 5.4.4, only these two extremes are shown.

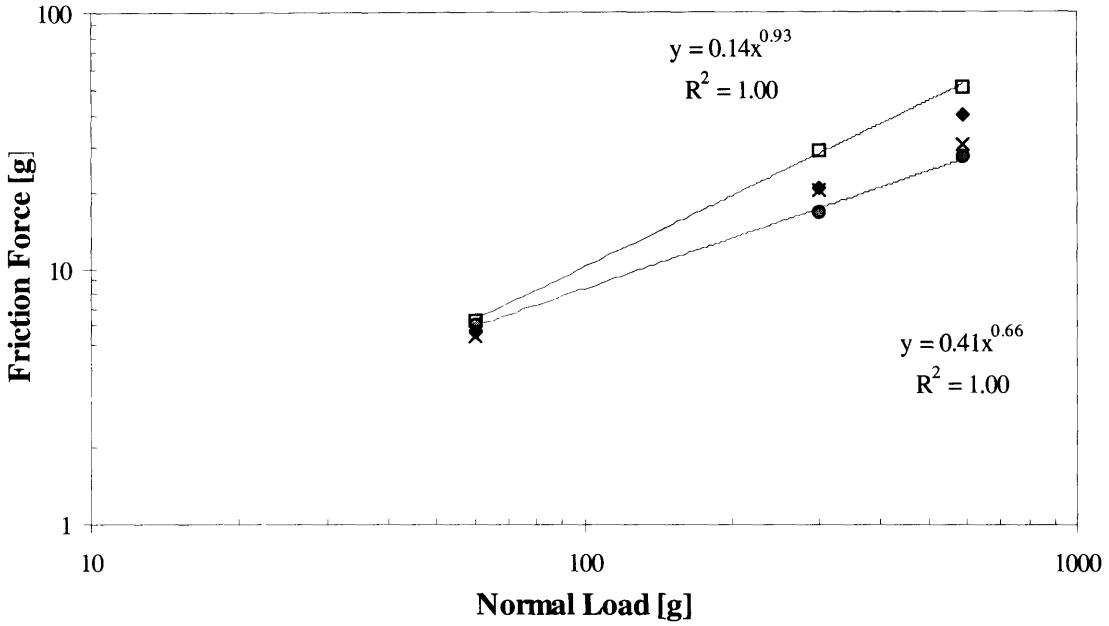

Fig. 5.4.4 Mean dynamic friction force versus normal load for PE on Co-Cr using four different lubricants Open squares represent the TKA case, black diamonds represent distilled water, "x" represents bovine serum, and gray circles represent the OA case. Error bars are not shown. Power law fit is given for the TKA case (upper line and equation) and the OA case (lower line and equation).

There is significance to the impact of load and pin size on friction. Specifically, both the magnitude of friction and the ability to distinguish among lubricants depends on these geometric parameters. This finding is further explained in section 5.9. For now, it suffices to say that the results of these tests supported the use of a 3 mm spherical pin under a load of 589 g, so these conditions were used for the remainder of the experiments.

5.5 Joint Fluid as a Lubricant

Now that a rapid tribological assay has been developed, this assay can be used to distinguish among lubricants in TJA. Specifically, the primary aim of this thesis is to determine whether different joint fluid samples affect the tribology of PE on Co-Cr. It was hypothesized that the tribology of PE on Co-Cr would be highly variable with joint fluid samples as lubricants.

5.5.1 Additional Materials

Seven synovial fluid samples were obtained from patients with OA during TKA surgery. Three joint fluid samples were obtained during revision TKA in other patients. All samples came from Brigham and Women's Hospital, New England Baptist Hospital, or Baw Beese Sports Medicine and Joint Care in accordance with a protocol approved by the Institutional Review Board and with the patients' informed consent. Patient information was obtained from medical records. The HA, protein, and phospholipid content of many of these fluid samples were reported in Chapter 4.

5.5.2 Statistical Methods

When sufficient fluid could be obtained, each Co-Cr disk was tested once under each lubrication condition (n = 6). Comparisons between mean coefficients of friction were performed using a two-tailed unpaired Student's t-test. These sample sizes were sufficient to determine, with 95% confidence, a 20% difference in coefficient of friction (with $\alpha = 0.05$ and $\beta = 0.05$) between two groups with a 10% coefficient of variation. Although friction was typically measured using six different Co-Cr disks, in some cases there was only sufficient fluid to evaluate two or four disks.

5.5.3 Results

The ten joint fluid samples tested exhibited a wide range of lubrication characteristics in the articulation of PE on Co-Cr, and could be fit into three groups (Table 5.5.1). Specifically, three of the ten samples enabled static and dynamic friction significantly less than distilled water ($p \leq 0.04$, dynamic; $p \leq 0.009$, static) and statistically equivalent to that of bovine serum. Three other samples enabled static and dynamic friction equivalent to water. For each of these samples, μ_d was greater than that of serum ($p \leq 0.002$). Static friction was greater than that of serum as well, but this difference was only statistically significant for the sample with sufficient fluid to test six times ($p = 0.0055$; $p = 0.07$ for $n = 4$ and $p = 0.15$ for $n = 2$). The four remaining samples performed *worse* than water. Under dynamic conditions, friction was significantly higher for these samples than for serum ($p < 0.0001$) or water ($p \leq 0.0062$). Static friction was also higher than that determined for serum ($p < 0.0005$), but not water. It should be noted that for several samples, the static friction force exceeded the range of the measuring apparatus (see Table 5.5.1), thus limiting the device's utility to distinguish among poor lubricants using static coefficient of friction.

5.5.4 Discussion

There was substantial variability in friction of PE on Co-Cr when different joint fluid samples were used as lubricants. Specifically, bovine serum and some joint fluid samples performed significantly better than distilled water. This finding indicates a component of some joint fluid samples and bovine serum that performs significantly better than water alone in the boundary lubrication of this couple. The component may not be same in these two fluids. Furthermore, the finding that some samples performed as well as distilled water, whereas other performed much worse, shows that the component of joint fluid that provides improved lubricity is either not universally present

in joint fluid or is variably blocked in some cases. This topic is discussed further in section 5.9.6.

This finding confirms the primary hypothesis of this thesis: joint fluid can affect the tribology of metal-on-PE articulations. It was hypothesized that the range would be similar to the difference between water and bovine serum, but here we find that the range quite exceeds that of water and serum. Thus, one would expect a range of wear rates exceeding the factor of three difference typically reported between water and bovine serum. It should be noted that the fractional difference in friction between water and serum lubrication was generally consistent with the difference between the "lubricating" and "non-lubricating" fractions of synovial fluid reported by Swann *et al.* for cartilage on cartilage.[37]

Table 5.5.1 Static and dynamic friction among joint fluid samples for PE on Co-Cr Joint fluid samples and other lubricants are ordered by increasing coefficient of dynamic friction (presented as mean ± standard deviation). The samples are further stratified into three groups. The first group of three samples (gray background) brings about coefficient of friction similar to that of bovine serum. The second group of three (plain background) brings about coefficient of friction similar to that of distilled water. The final group brings of four (gray background) brings about friction significantly higher than that of water. ID = Study ID. N/A = Not applicable or not available. PTA = post-traumatic arthritis. One of six output curves for Study ID H01 is given above in Fig. 5.4.1. [a]Tested in initial group, under 3 loads, with 3 different pin sizes. [b]Two of six samples exceeded the maximum friction force measurable on the apparatus. The calculations are based upon a coefficient of static friction of 0.19 for these measurements. [c]Three of six samples exceeded the maximum friction force measurable on the apparatus. The calculations are based upon a coefficient of static friction of 0.19 for these measurements.

ID	*Description*	*Diagnosis*	*n*	μ_s	μ_d
018	Primary TKA	OA	2[a]	0.085 ± 0.008	0.046 ± 0.004
	Bovine serum	N/A	12	0.12 ± 0.02	0.054 ± 0.006
H16	Primary TKA	OA	6	0.12 ± 0.02	0.053 ± 0.004
H01	Primary TKA	OA	6	0.12 ± 0.01	0.055 ± 0.004
	Distilled Water	N/A	6	0.16 ± 0.03[b]	0.067 ± 0.012
H23	Primary TKA	OA	6	0.16 ± 0.02	0.068 ± 0.005
H17	Primary TKA	OA	4	0.14 ± 0.04	0.070 ± 0.008
172	Revision, 3 years post TKA PE wear	PTA	2	0.14 ± 0.03	0.077 ± 0.008
H20	Primary TKA	PTA	2	0.16 ± 0.001	0.085 ± 0.007
201	Revision, 8 years post TKA PE wear & osteolysis	OA	6[a]	0.18 ± 0.02[c]	0.087 ± 0.004
H18	Primary TKA	OA	6	0.16 ± 0.02[b]	0.088 ± 0.008
047	Revision TKA (Instability)	N/A	2	0.15 ± 0.01	0.091 ± 0.002

5.6 Tribology of Joint Fluid Components

Having established the variability of the tribology of TJA under joint fluid lubrication, the effects of three individual components of joint fluid were compared. It was hypothesized that the variability in friction between PE and Co-Cr could be

explained by variation in HA, protein, and phospholipid within physiological ranges. These tests were conducted using PE articulating on both Co-Cr and Ox-Zr. In each case, a sample size of five was used. Additionally, both couples were evaluated under bovine serum and saline lubrication conditions. Bovine serum was evaluated with a sample size of six for Ox-Zr and twelve for Co-Cr (six during the initial determination of methods, and six in concert with Ox-Zr evaluation, to ensure repeatability of measurement).

5.6.1 Additional Materials and Methods

The lubricants used for this portion of the study (except bovine serum) were based on Dulbecco's phosphate-buffered saline (PBS) (catalog number 14191-144, lot number 1152114, Invitrogen Corporation, Grand Island, NY). In evaluating the tribological effect of individual components of joint fluid, HA, protein, or phospholipid was added to PBS. These components were added based upon the physiological ranges of these components in TJA joint fluid reported in Chapter 4. The HA added was sodium hyaluronate with viscosity average molecular weight 1.76 x 10^6 Da (catalog number 80190, lot number P9412-2, Lifecore Biomedical, Chaska, MN). The two physiological solutions made contained 0.66 and 2.45 mg/ml HA. The phospholipid added was DPPC (part number P-0763, lot number 90K5234, Sigma-Aldrich, St. Louis, MO). The two physiological solutions made contained 0.42 and 0.97 mg/ml DPPC. The proteins added were albumin (Grade III egg albumin, part number A-5378, lot number 68F-8150, Sigma-Aldrich, St. Louis, MO) and γ-globulin (bovine, from plasma, part number G-7516, lot number 91K7070, Sigma-Aldrich, St. Louis, MO). These were added in a 2:1 albumin:γ-globulin ratio to approximate the proteins in joint fluid using a small number of proteins.[38] One solution contained 6.8 mg/ml albumin and 3.4 mg/ml γ-globulin, and the other contained 33 mg/ml albumin and 16 mg/ml γ-globulin.

Experimental Protocol

Generally, the experimental protocol used in this section was identical to that used in the remainder of the chapter. Several steps were taken to ensure the repeatability of measurement. Specifically, to ensure that the system as a whole did not change with time, PE on Co-Cr was tested under bovine serum lubrication on two separate occasions, each with a sample size of six. Furthermore, to ensure that differences between groups were not due to differences between PE pins, each series of tests using one pin and six disks employed PBS for one test and another lubricant for the other five. The order of the tests (*e.g.*, Ox-Zr then Co-Cr, PBS then PBS plus component) was randomized to determine whether changes to the PE pin during the test affected the friction measured in subsequent tests. There was no significant effect of the order in which tests were run, and the friction measured for bovine serum was the same in both groups of six, thus demonstrating the robustness of the test and justifying the use of a single pin for six tests.

5.6.2 Results

When lubricated by PBS, no difference was demonstrated between Co-Cr and Ox-Zr (Fig. 5.6.1), though for both μ_d and μ_s, a trend toward reduced friction using Ox-Zr was clear. Statistical significance was prevented by high standard deviation in Ox-Zr results under PBS lubrication. Lubrication by bovine serum reduced friction in both

couples, both statically and dynamically, but the difference was only statistically significant in μ_d of PE on Co-Cr ($p = 0.025$). Nonetheless, variability in Ox-Zr was much reduced by serum lubrication, and so the 15% reduction in friction for PE on Ox-Zr compared to PE on Co-Cr was highly significant (μ_d, $p < 0.009$; μ_s, $p = 0.01$).

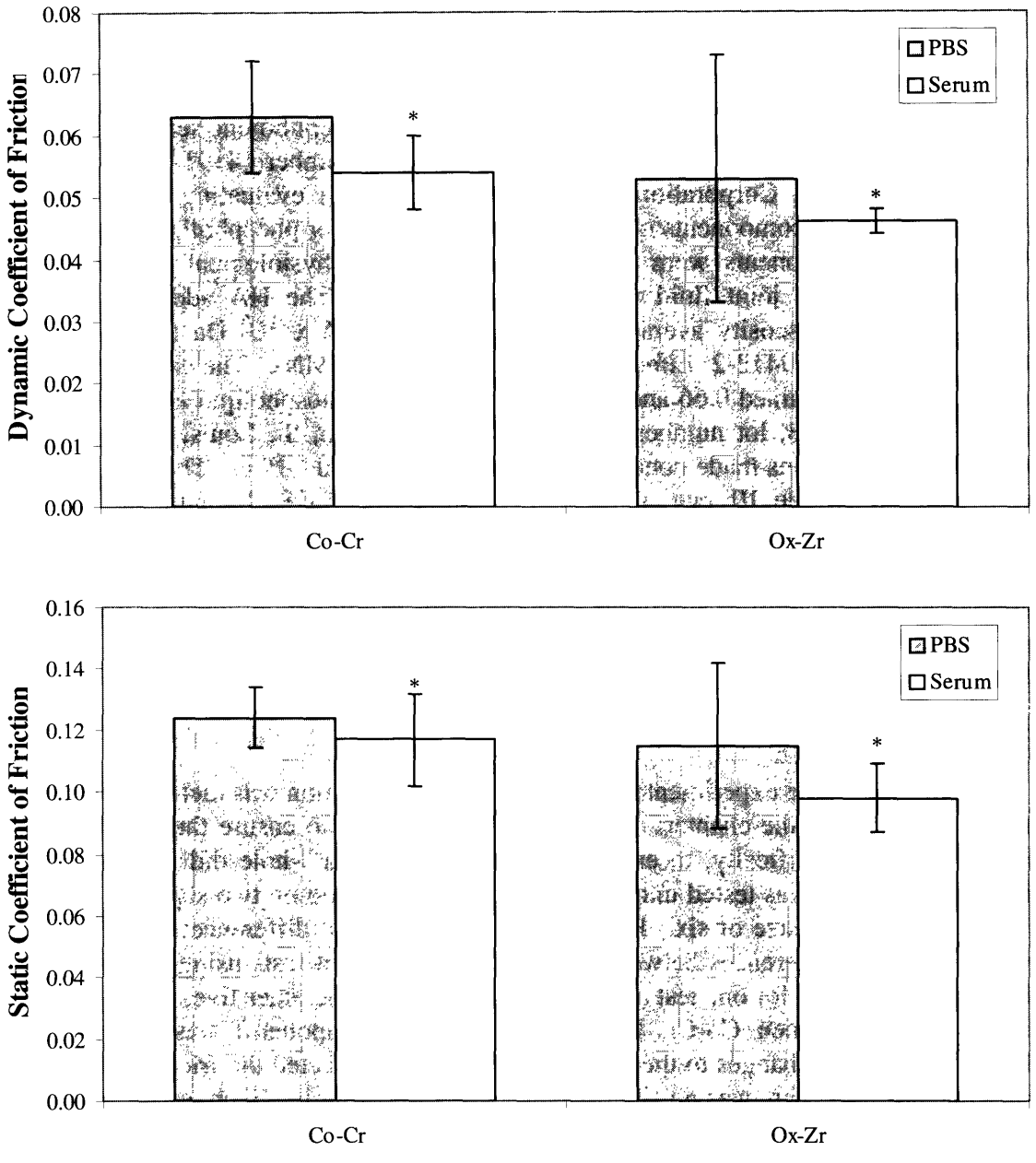

Fig. 5.6.1 Dynamic (above) and static (below) friction between PE and metal when lubricated by PBS and bovine serum Dark columns indicate friction under PBS lubrication, and light columns indicate friction under bovine serum lubrication. Error bars represent standard deviation. Asterisks indicate statistically significant differences between Co-Cr and Ox-Zr. The high standard deviation in Ox-Zr under PBS lubrication prevented a statistically significant difference between Co-Cr and Ox-Zr under these conditions.

When compared to PBS, the addition of HA and protein had significant effects on dynamic friction when PE articulated on Co-Cr (Table 5.6.1 and Fig. 5.6.2). Specifically, HA and protein both increased friction in the couple. For protein, adding either physiological amount resulted in a 24% increase in μ_d ($p < 0.006$). In μ_s, only a high physiological concentration of protein increased friction significantly ($p = 0.003$) (20% increase). A difference was also shown between the high physiological and low physiological protein groups, with high protein content increasing static friction ($p = 0.025$). When added at high physiological concentration, HA increased μ_d by 17% above that of PBS alone ($p = 0.022$). The effect of HA at low physiological concentration was consistent with a dose-dependent response, but the increase in friction was not statistically significant. For μ_s friction, the same trend toward higher friction at high HA concentration was seen, but statistical significance was not reached (e.g., $p = 0.075$, high physiological concentration versus PBS). There was no effect of phospholipid on the friction of PE on Co-Cr at either low or high concentration. These data are also presented graphically for PE on Co-Cr in the dynamic case only in Fig. 5.6.2.

Table 5.6.1: Static and dynamic coefficients of friction of PE on two different metals with various lubricants Differences that are statistically significant, when compared to PBS, are shaded in dark gray, and discussed in the text. Differences between Co-Cr and Ox-Zr are shaded in light gray in the Ox-Zr column.

Lubricant	*Co-Cr*		*Ox-Zr*	
	μ_d	μ_s	μ_d	μ_s
PBS	0.063±0.009	0.124±0.010	0.053±0.020	0.115±0.027
PBS + 0.66 mg/ml HA	0.069±0.009	0.127±0.022	0.067±0.017	0.141±0.028
PBS + 2.45 mg/ml HA		0.141±0.011	0.055±0.017	0.125±0.030
PBS + 0.42 mg/ml DPPC	0.064±0.008	0.139±0.026	0.044±0.006	0.101±0.010
PBS + 0.97 mg/ml DPPC	0.061±0.004	0.121±0.017	0.048±0.006	0.096±0.023
PBS + 10 mg/ml protein		0.131±0.015	0.050±0.002	0.110±0.011
PBS + 50 mg/ml protein			0.054±0.002	0.113±0.014

In PE on Ox-Zr articulation, the components of joint fluid had little effect on friction. There was no effect of HA on static or dynamic friction. No statistically significant effects of phospholipid or protein concentration on the mean frictional coefficients were found. Both components did increase the repeatability of the data (*i.e.*, decreased the coefficient of variation) relative to PBS, however. The decreased variability in dynamic friction was statistically significant for both protein and phospholipid at high and low concentration (for each group, $p < 0.006$). The decreased variability in static friction approached statistical significance, but was not demonstrated in all groups (for all groups, $p < 0.09$). That is to say, the effect of phospholipid and protein on the friction between the PE and Ox-Zr couple was to decrease the intra-sample variability relative to PBS. The variability in PE on Co-Cr when lubricated by protein or phospholipid containing lubricants was similar to that in PE on Ox-Zr, but it represented no decrease relative to PE on Co-Cr lubricated by PBS.

In addition, the results of PE on Co-Cr lubricated by joint fluid were stratified by total protein, HA, and phospholipid content, as presented in Chapter 4 (Appendix L).

Regression analysis revealed a significant (p = 0.012) but very weak relationship (R^2 = 0.17 for linear regression) between HA concentration and μ_d, such that increased HA led to increased friction. This relationship was consistent with the one that would be expected from the data regarding PBS plus HA, but the relationship was mostly due to one outlying data point (high friction and high HA concentration). There was also a weak relationship between protein concentration and μ_d (p = 0.050, R^2 = 0.11 for linear regression), but it showed a decrease in coefficient of friction as protein increased. This result was inconsistent with the results for PBS plus protein, which demonstrated an increase in friction as albumin and γ-globulin content increased. No relationship was found between friction and phospholipid.

Due to the increased friction of PE on Co-Cr under HA and protein lubrication, and due to the reduced variability of PE on Ox-Zr friction under protein and phospholipid lubrication, there were several statistically significant differences between Co-Cr and Ox-Zr as counterfaces for PE (Table 5.6.1 above and Fig. 5.6.2 below). For example, under protein lubrication, the use of Ox-Zr instead of Co-Cr resulted in a statistically significant reduction in friction for both low concentration (μ_d, $p < 0.0001$; μ_s, p = 0.038) and high concentration (μ_d, $p < 0.0001$; μ_s, p = 0.0012). Phospholipid brought about similar differences (high concentration: μ_d, p = 0.0034; low concentration: μ_d, p = 0.0021, μ_s, p = 0.016), though static friction was not significantly different at low concentration (p = 0.087). Under HA lubrication, the only statistically significant difference was μ_d at high concentration (p = 0.04).

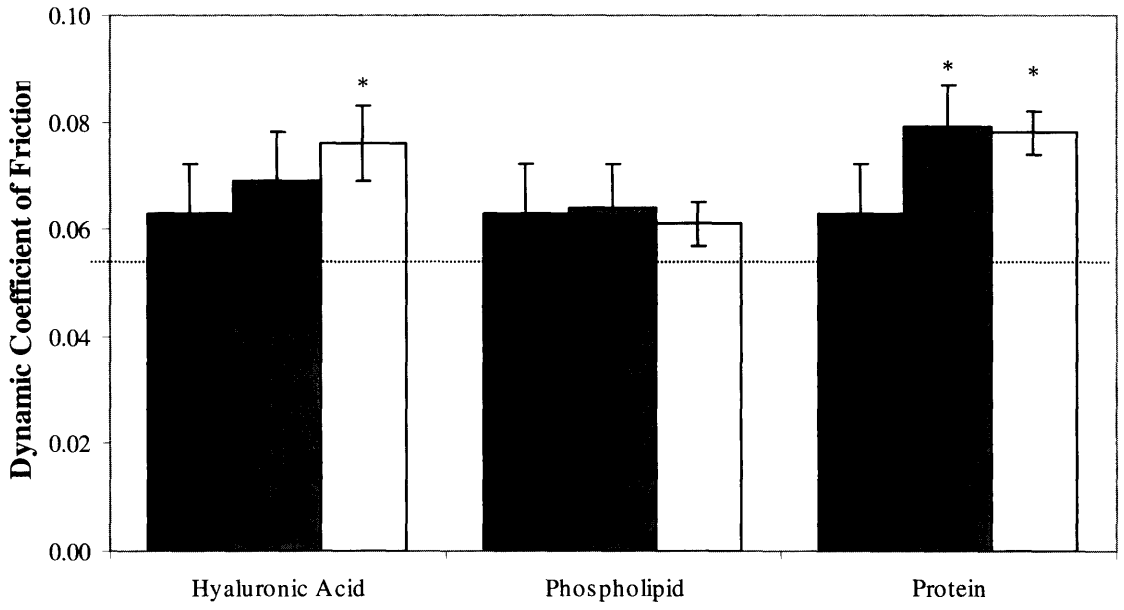

Fig. 5.6.2 Dynamic friction between PE and Co-Cr when lubricated by various components of joint fluid These are the same results presented in Table 5.6.1 in graphical form for simplified comparison. Dark columns indicate friction under PBS lubrication, gray columns indicate PBS plus a low physiological amount of each lubricant; and light columns indicate PBS plus a high physiological amount of each lubricant. Error bars represent standard deviation. Asterisks indicate a group that is statistically significantly different from PBS ($p < 0.05$). The dotted line indicates the mean dynamic coefficient of friction of bovine serum, shown for reference.

5.6.4 Discussion: Co-Cr versus Ox-Zr

When lubricated by bovine serum, PE on Ox-Zr had a lower coefficient of friction than PE on Co-Cr. This finding is consistent with laboratory studies showing reduced wear rates in Ox-Zr on PE relative to Co-Cr on PE when lubricated by serum under abrasive[39] and non-abrasive[40] conditions. The explanations offered for this difference include improved wettability of Ox-Zr (reducing adhesive wear) and the increased hardness of Ox-Zr relative to Co-Cr (reducing the roughening of the metal that leads to rapid abrasive wear).

Furthermore, both HA and protein increased the friction of PE on Co-Cr, but had no effect on the friction of PE on Ox-Zr. Each component of joint fluid studied increased friction of PE on Co-Cr relative to PE on Ox-Zr in a statistically significant manner. Thus, although no difference was demonstrated between Co-Cr and Ox-Zr under PBS lubrication, bovine serum and three components of joint fluid suggest that Ox-Zr on PE may provide better tribology than PE on Co-Cr *in vivo*. The worst joint fluid samples generated friction consistent with that of PBS plus protein plus HA when lubricating PE on Co-Cr. These components that negatively influenced the tribology of PE on Co-Cr did not negatively influence the tribology of PE on Ox-Zr. Thus, the frictional difference between Co-Cr and Ox-Zr was greatest in the cases that likely would produce the most wear in PE on Co-Cr articulation. These data support the hypothesis that, for cases in which PE on Co-Cr would result in high wear, Ox-Zr represents a significant tribological improvement.

5.6.5 Discussion: Components of Joint Fluid

The negative effect of protein on friction between PE and Co-Cr is somewhat surprising. Since proteins in bovine serum are often credited with causing the wear rates and morphology similar to clinical findings (*e.g.*, McKellop *et al.*[1]), it might be expected that a protein-containing lubricant would result in friction similar to that of bovine serum. In this study, the opposite effect on friction was shown. Previously, other studies had compared bovine serum to albumin and γ-globulin as lubricants for metal-on-PE. In a metal on plastic hip simulator study, albumin generated friction similar to that of saline, whereas serum produced friction similar to that of synovial fluid.[34] In bidirectional experiments, Saikko and Ahlroos found a higher *initial* wear rate for stainless steel on PE when lubricated with serum, as compared with albumin or γ-globulin, though after many cycles, the wear rates appeared to equalize.[41] In unidirectional experiments, others have found protein-containing lubricants to perform differently than bovine serum in PE on stainless steel.[14,42] In conjunction with the present experiments, these studies all suggest that albumin and γ-globulin are not the components of bovine serum that bring about reduced friction and wear relative to saline. Extending this finding to joint fluid, it is clear that albumin and γ-globulin are not the components of joint fluid that in some cases bring about reduced friction.

It should be noted that these studies employed a stainless steel counterface, and, as the present study demonstrates, the importance of individual components may depend on the counterface on which PE articulates. It is not demonstrated by these results that any of the components of joint fluid have a detrimental effect on the tribology of Ox-Zr

on PE. If any effect of these components is suggested for the Ox-Zr on PE couple, it is that protein and phospholipid make the friction less variable relative to saline lubrication.

HA likewise increased friction between PE and Co-Cr. Although it has not been suggested that HA provides a boundary lubrication function in this articulation, it was not expected that its presence would *hinder* lubrication. A previous unidirectional pin-on-flat wear test evaluating PE on an alumina surface found HA to decrease both friction and wear, though the authors attributed this finding to mixed or hydrodynamic lubrication, rather than boundary lubrication.[14] The geometry of the present study precludes fluid film lubrication, and isolates the boundary lubricating effect of HA.

DPPC has previously been shown to lubricate soft tissue physiological articulations under low load[43] as well as synthetic couples such as glass on cotton[44] and metal-on-metal.[45] There is also evidence for its role in lubricating synovial joints.[46,47] Furthermore, DPPC and other phospholipids have been found to decrease the wear of PE on stainless steel in unidirectional[48] and multidirectional[49] POF tests. In both cases, wear decreased to an immeasurably low value when lubricated by phospholipid. The present experiments do not support this finding in PE on Co-Cr or PE on Ox-Zr, however. No effect of phospholipid was shown, positive or negative, in either articulating couple.

In a hip simulator, the addition of phospholipid to a protein-based lubricant significantly decreased the wear of PE articulating on Co-Cr. Strangely, this low wear rate was not replicated by bovine serum, a protein-based lubricant containing phospholipid.[50] The results of this study are not replicated here, but the lubrication achieved by bovine serum is shown not to be due to phospholipid.

Furthermore, these findings suggest that the variable tribology found in PE on Co-Cr lubricated by joint fluid does not arise from variation in total protein, HA, or phospholipid content. Specifically, the low friction sometimes characteristic of this articulation cannot be explained by HA, albumin, γ-globulin, or phospholipid. Interestingly, it now becomes much less clear what component of bovine serum provides its well-documented lubricating advantage over water for this articulating pair. Neither total protein nor phospholipids (both formerly likely candidates) appear to be appropriate choices.

The regression analysis correlating concentration of HA and phospholipid with friction was consistent with the result of the addition of these components to PBS. The relationship with protein was opposite the one that would have been expected. Due to the small sample size and relatively large number of covariates, regression analysis was not sufficient to draw conclusions about the effect of these variables.

5.7 Synthetic Joint Fluids

To further establish the connection (or disconnection) between joint fluid composition and tribology, two lubricants were synthesized to consist of the same amount of protein, phospholipid, and HA as two synovial fluid samples that exhibited very different frictional characteristics. One was chosen from the lowest friction group (equivalent to bovine serum), and one from the middle friction group (equivalent to water). These samples were synthesized in the same fashion as individual joint fluid components. The composition of these samples, as well as that of their synthetic counterparts, is given in Table 5.7.1. Both samples had compositions typical of joint

fluid samples from patients undergoing TKA, though there were meaningful differences between the samples in both HA and phospholipid concentration.

Table 5.7.1: Composition of two synovial fluid samples and their synthesized counterparts All concentrations are given in mg/ml. In both joint fluid cases, the mass average molecular weight of HA was 1.8 MDa. Both samples were obtained from OA patients at TKA. Case 1= Study ID 018; Case 2 = Study ID H17.

Description	*Protein*	*Albumin*	*γ-globulin*	*HA*	*Phospholipid*
Case 1	31.4	N/A	N/A	1.16	0.48
Synthetic Case 1	31.4	20.8	10.6	1.26	0.51
Case 2	29.8	N/A	N/A	0.86	0.78
Synthetic Case 2	30.5	20.5	10.0	0.82	0.74

In both cases, the synthetic fluid performed significantly worse than natural joint fluid in lubricating PE on Co-Cr (Fig. 5.7.1). For the first case, a difference was observed for both μ_d and μ_s ($p < 0.0001$, $p = 0.0042$, respectively) whereas, for the second case, a difference was only evident in mean dynamic friction ($p = 0.0003$). Since in all measurements, static friction was within the measurable range of the device, no difference between the groups was obscured by the limits of the device. No difference was demonstrated between the two synthetic joint fluid samples despite the differences in phospholipid and HA concentration.

The comparison between "synthetic" and "real" joint fluid samples confirms what is suggested by the evaluation of individual components. The presence of HA and protein content can explain why some samples perform worse than water, but cannot explain why some joint fluid samples perform better than water, or why bovine serum performs better than water (bovine serum contains both protein and phospholipid, but no HA). A component of joint fluid not evaluated must be responsible for the difference between these samples, as well as the difference between natural and synthetic joint fluid samples.

Furthermore, the coefficients of friction observed between PE and Co-Cr with synthetic lubricants were higher than those recorded in PBS plus HA and PBS plus protein. These findings suggests that protein and HA have an additive effect, in that they both interfere with boundary lubrication in this articulation; when present in physiological concentrations, the presence of both is worse than the presence of either component alone.

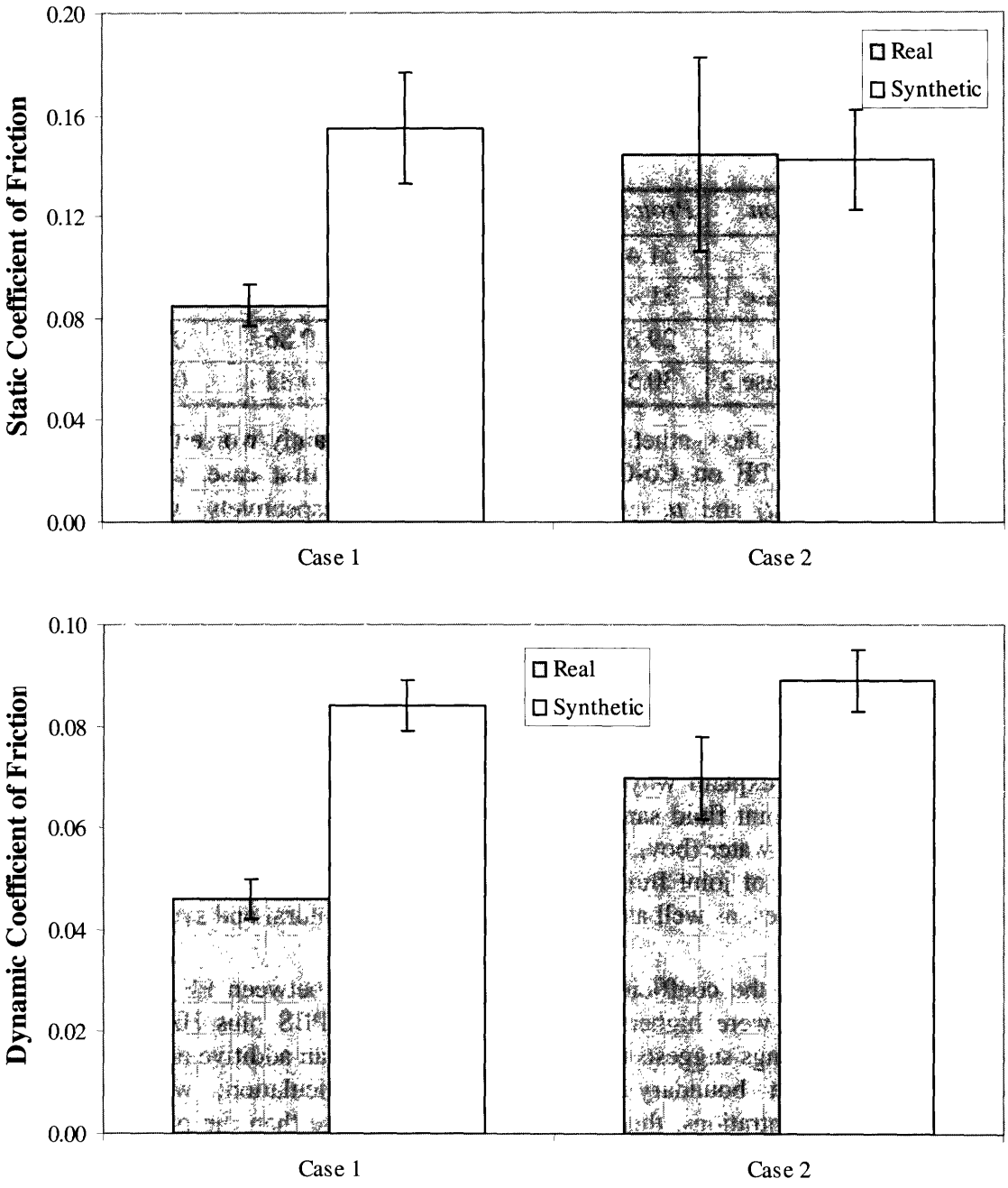

Fig. 5.7.1 Static (above) and dynamic (below) friction of two joint fluid samples and synthetic counterparts Synthetic joint fluid samples consisted of PBS plus the same HA, protein, and phospholipid concentrations as natural joint fluid. Dark columns represent results from real joint fluid samples; light columns indicate results from synthetic joint fluid samples. Error bars represent standard deviation. Both true joint fluid samples performed better than their synthetic counterparts in dynamic friction ($p \leq 0.0003$); in static friction, only Case 1 performed better ($p = 0.004$).

5.8 Protease Digestion of Bovine Serum

The final objective of this study was to begin to identify what component or components of joint fluid and bovine serum lead to the low coefficient of friction

between Co-Cr and PE. A study designed to clearly identify this component in joint fluid would be an extensive undertaking and is not within the scope of this thesis. Nonetheless, it is a fitting conclusion to this study to take initial steps towards identifying the critical boundary lubricants.

In the studies of this chapter, the use of albumin and γ-globulin in a 2:1 ratio was based upon a previous report suggesting that half of the protein in joint fluid is albumin, and one quarter is γ-globulin.[38] Other reports give a higher albumin to γ-globulin ratio (*e.g.*, Walker *et al.*[51] and McCarty *et al.*[52]). In any case, a substantial portion of the protein in joint fluid is neither albumin nor γ-globulin. This unaccounted protein may be responsible for the decreased coefficient of friction under bovine serum or joint fluid lubrication. The fact that a lubricating protein was found responsible for the tribology of cartilaginous articulation under synovial fluid lubrication supports the hypothesis that a protein can perform this function.

Ideally, one would examine joint fluid samples to determine the lubricating component of joint fluid. Given the small quantities of joint fluid available, however, and its variable tribological performance, it was appropriate in a preliminary study to evaluate bovine serum instead. To destroy the proteins in a sample of bovine serum, 3.5 mg proteinase K (catalog number P-6556, lot number 081K8623, Sigma-Aldrich, St. Louis, MO) were added to 10 ml of bovine serum from the same source as described in section 5.4.1. The mixture was shaken vigorously then heated to 37°C for 16 hours. Afterwards, it was cooled to room temperature. The digested bovine serum was then used as a lubricant for PE on Co-Cr according to the same protocol as used above.

The digested bovine serum samples performed significantly worse than undigested bovine serum samples (as in Table 5.5.1), both statically ($\mu_s = 0.165 \pm 0.020$, $p = 0.0018$) and dynamically ($\mu_d = 0.095 \pm 0.003$, $p < 0.0001$). Mean friction for digested bovine serum was higher still than water, but the difference was only statistically significant in dynamic friction ($p < 0.0001$). A difference in static friction may have been masked by the saturation at high friction, which did occur in one case with proteinase digestion. In dynamic friction, bovine serum digested by protease was consistent with the worst group of joint fluid samples described in section 5.5.

These results indicate that one or more protein components of bovine serum are necessary for the lubrication of PE on Co-Cr. Furthermore, the increase in friction to a level comparable to that of albumin and γ-globulin lubrication suggests that digested proteins interfere with lubrication in a fashion comparable to that of these poorly-lubricating proteins.

Ideally, these results would be compared to control serum measurements conducted simultaneously. Two such tests were conducted using bovine serum without protease digestion. These two serum samples generated higher friction than previous serum measurements, both statically and dynamically. It is not clear why this occurred; perhaps some proteins in the serum changed during the month it was in refrigerated storage between earlier tests and the present test. Nonetheless, the increase in friction under dynamic conditions was still highly statistically significant for the digested bovine serum ($p < 0.005$). The increase in friction in the static case was not statistically significant, but this was likely due to the small sample size of both groups ($n = 2$ and $n = 4$). A quite large disparity (0.043) in mean μ_s would have been necessary to

determine a difference between groups with 95% confidence using these sample sizes. Given the dynamic result in conjunction with the difference from baseline bovine serum data, it is justified to interpret the increase in friction as indicative of the destruction of one or more lubricating components of bovine serum by the proteolytic protocol. Nonetheless, repeating these tests with a larger sample size would demonstrate this relationship more conclusively. It should be noted that heating bovine serum at 37°C for 16 hours may have degraded the component(s) responsible for lubrication. This hypothesis could be tested further by heating a sample of bovine serum without protease, and examining its effect on friction.

5.9 A Conceptual Model for the Tribology of PE-on-Metal

The results of this chapter, though meaningful, leave a gap between friction and wear. Although the differences in friction measured in this study are relevant to the tribology of TJA, greater importance is tied to wear than friction. There are both theoretical and empirical associations between friction and wear, and these can be carefully applied to the present results. For example, the friction generated in boundary lubricated contact can be related to the energy-dissipative processes associated with adhesive and abrasive wear of PE as per section 5.1.1. Additionally, the next chapter provides additional evidence (above that already in the literature) showing a connection between friction and wear in this couple. Thus, some arguments and data are presented in this thesis to connect friction and wear, but a complete framework for tribology of TJA is lacking. The subsequent discussion presents a conceptual framework for the present work so that it may be appropriately applied to the tribology of TJA.

5.9.1 Introducing the Illustration

The conceptual model that is presented is illustrated schematically below in Fig. 5.9.1. In this schematic, the PE surface (white) is shown on top. The metal surface (gray) is shown below. In the figure, the surfaces interacting are both essentially flat, as in POF wear tests, though they have some roughness. Later, the influence of replacing the PE surface with a sphere is discussed. For simplicity, only the asperities on the metal surface are shown, though in reality, there are also asperities on the PE surface. Some asperities are shown as rounded, while others are sharp; this is done to indicate the presence of both adhesive and abrasive wear, and does not represent the actual topography of the metal surface. In the space between the surfaces, a boundary lubricant is shown. Specifically, the boundary lubricant has the form of a phospholipid, consistent with the form of good boundary lubricants for metal-on-metal articulations. The use of phospholipid as a boundary lubricant does not indicate support for phospholipid as a boundary lubricant in metal-on-PE articulations. As discussed above, phospholipid had no effect on the tribology of the PE on Co-Cr articulation. The characteristics of an excellent boundary lubricant for metal-on-metal articulation differ from those for PE-on-metal. These differences are discussed below (see section 5.9.6).

Damage to the PE surface and PE wear particles are not shown in the interface. In the POF wear tests of Chapter 6, several cubic millimeters of PE particles are produced every million cycles. If the average particle (by mass, not number) is 1 μm on a side, the average cycle produces thousands of PE wear particles. These are largely removed by the sliding motion, and have relatively little affect on the surface once generated (unlike

particles in constrained bearings, which remain in the articulation and lead to the extremes of solid lubrication and seizure[53]). These particles are not produced in a single cycle, however. The PE surface is constantly changing, with wear particles in the process of being generated and released. A true depiction of the PE surface would show roughness as well as damage leading to PE particle generation. Since the PE surface is so compliant relative to the metal counterface, the topography of the PE surface has little effect on the rate of wear particle generation. This argument is supported in Chapter 6 by an analysis of the change in PE wear rate with time as the pin surface topography changes. Consequently, for this first order approximation, ignoring the effects of damage to the PE surface as well as newly generated wear particles is appropriate.

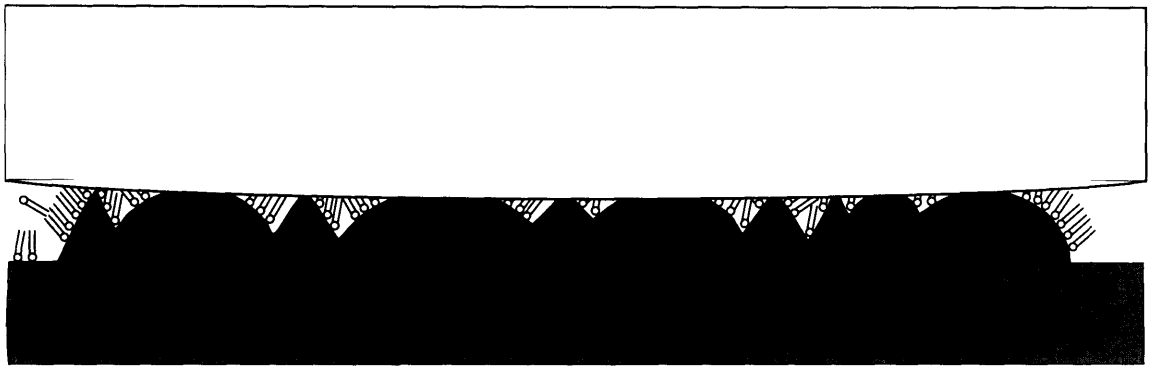

Fig 5.9.1 Schematic of PE articulating on metal A description of this schematic is given in the text above. This schematic is used as an illustrative baseline for discussing the tribology of PE-on-metal.

5.9.2 Friction in Boundary Lubrication

The frictional force generated in boundary lubricated metal-on-PE contact can be broken into two components: the portion generated by wear processes, such as adhesion or plowing of PE, and the portion generated by shearing adsorbed boundary lubricant. Breaking each component into an average shear stress and an area of action, we find

$$F_f = \tau_l A_l + \tau_w A_r, \qquad \textit{Equation 5.9.1}$$

where F_f is the total frictional force, τ_l is the shear stress required to shear the boundary lubricant, τ_w is the shear stress associated with wear processes, A_l is the effective area of lubricated contact, and A_r is the effective area of surface-surface contact (which could also be called the real area of contact). This treatment has been used in metal-on-metal couples, as in Dintenfass *et al.*[54] and Komvopoulos *et al.*,[21] but is valid for PE-on-metal couples as well. (Other models for metal-on-polymer wear have been proposed (e.g., Hsu *et al.*[25]), but these refer to other types of articulations, and are not appropriate for TJA.) Equation 5.9.1 is identical to one presented by Komvopoulos *et al.*, except for subscripts, which have been modified for the present discussion. A_l and A_r are shown schematically in Fig. 5.9.2. It is important to note that, for flat-on-flat articulation, the sum $A_l + A_r$ is less than A_a, the apparent contact area. The additional area comprising A_a does not bear load.

Within this schematic understanding, both A_l and A_r bear load. A_l does so through the load-bearing capability of a boundary lubricant, whereas the materials themselves bear the load in A_r. With regard to friction, however, the direct contribution of A_l is

minimal. In most articulations, $\tau_w >> \tau_l$ as suggested by Komvopoulos *et al.* for metal-on-metal boundary lubrication.[21] Consequently, the lubrication term is negligible relative to the wearing term with regard to friction, and

$$F_f \sim \tau_w A_r. \qquad \textit{Equation 5.9.2}$$

This relationship between friction and real area of contact is commonly used in the tribology literature, and was discussed in section 2.4.1.

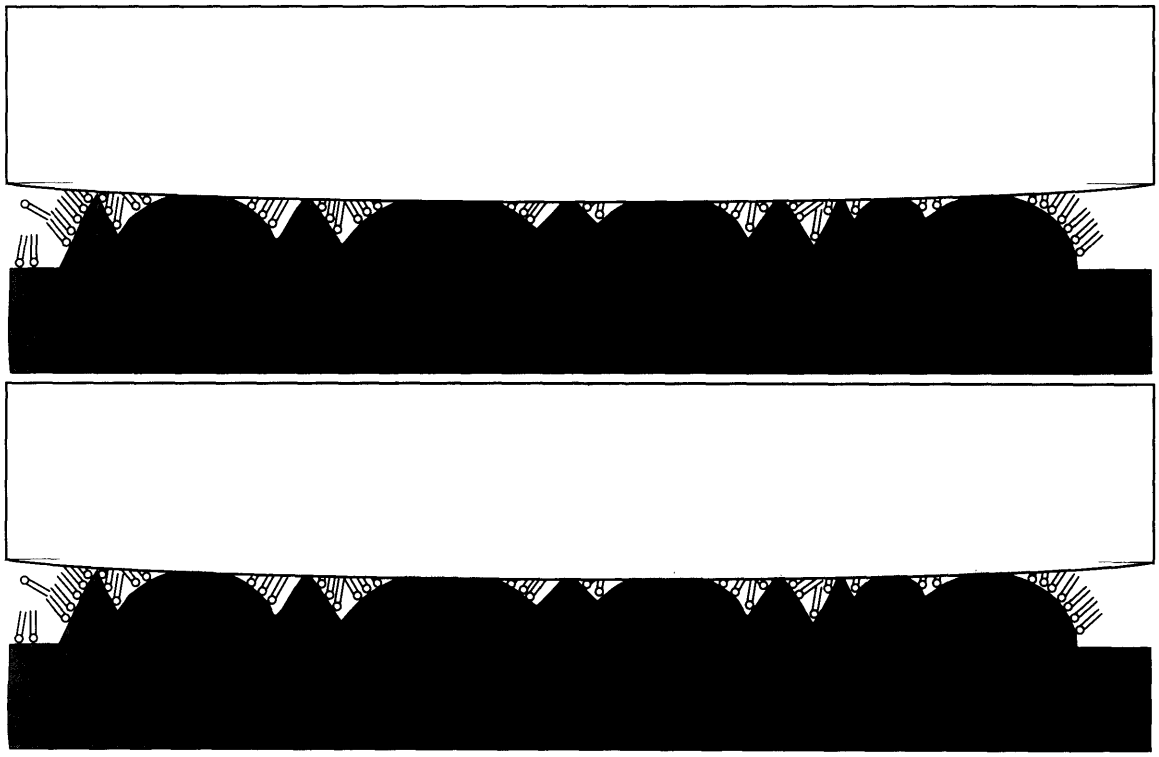

Fig 5.9.2 Schematic of A_l (above) and A_r (below) in boundary lubrication of PE-on-metal Expanding on the schematic of Fig. 7.3.1, the shaded areas in the above illustration indicate places in which interaction between the surfaces is influenced by boundary lubricant as in Equation 7.3.1. The shaded areas in the lower illustration indicate places in which interaction between the surfaces is not greatly influenced by boundary lubricant as in Equation 7.3.1. These interactions lead to the generation of wear particles. There exists an additional area over which the surfaces do not interact, so the sum of A_l and A_r is less than A_a. Fig. 5.9.3 improves upon this illustration by considering deformation of the PE surface.

It should be evident already that this analysis is only semi-quantitative. Although an equation has been generated, the variables in the equation are conceptual in nature. For example, the areas shaded in Fig. 5.9.2 are not rigidly defined. I do not suggest that wear is as simple as "lubricated" area and "not lubricated" area. Various areas of contact are likely to contribute to wear variably. Certain asperities that are particularly sharp may lead to much higher wear than smoother asperities that may engage more area of contact, for example. Additionally, since the PE surface is not smooth as shown in the figures, A_r and A_l are not likely constant during articulation. For the purposes of a schematic framework, however, it is sufficient to consider two effective "lubricated" and "not lubricated" areas.

5.9.3 Plastic and Elastic Deformation

In the POF case, it may be difficult to establish A_l and A_r quantitatively. Both depend on the roughness of the surfaces, compliance of the surfaces, and A_a. For hard-on-hard articulations, such as in metal-on-metal, relatively little load is borne by elastic deformation. After a small amount of elastic deformation, asperities deform plastically, and bear load according to the definition of hardness:

$$A = W / H. \qquad \textit{Equation 5.9.3}$$

Typical discussions in metal-on-metal contact ignore the effect of a boundary lubricant in this load-bearing role, assuming $A \sim A_r$ (e.g., Stachowiak *et al.*[22]) rather than

$$A = A_r + A_l, \qquad \textit{Equation 5.9.4}$$

a more appropriate relation for lubricated PE-on-metal. Combining Equations 5.9.2 and 5.9.3 leads to a proportional relationship between F_f and W. Taking the ratio of these, the source of "coefficient" of friction is found. It is clear from the data of this chapter that such a linear relationship does not exist between friction and normal load in this articulation.

Other types of contact, such as sphere on flat, generate well-defined areas of contact, as described in section 5.3.2. In this case, the contact area is given as described in section 5.3.2. This occurs for both hard-on-hard and hard-on-soft articulations. Considering the area of contact in conjunction with the schematic of 5.9.2, it is appropriate to use the contact area of Equation 5.9.4, rather than the real area of contact as typically described in Hertzian contact stress analysis. (More often, Hertzian contact uses A_r, but Hertzian contact assumes no lubricant. The presence of a lubricant, combined with the conformity of surfaces, justifies the modification of the Hertzian contact equation.) Thus, the governing equation in this case would be

$$A = A_r + A_l = C \times W^{2/3}. \qquad \textit{Equation 5.9.5}$$

Empirically, the friction of metal-on-PE typically results in a power law relationship ($F_f \sim W^n$), where $0.67 \leq n \leq 1$. Considering this result in conjunction with Equation 5.9.2, this relationship suggests a mixture of elastic and plastic deformation (section 5.3.2 and Equation 5.9.3). This same result has been found even for POF articulations under certain loading conditions,[55,56] Some studies have supported elastic deformation at low loads and plastic deformation at higher loads,[55] but the definition of high and low loads must depend on the geometry and articulating conditions. This relationship is consistent with the present friction data regarding the effect of pin size and normal load. Specifically, the effect of normal load was consistent with the above power law relationship, and a greater decrease in coefficient of friction was found using a spherical pin (elastically-dominated contact) as compared to a flat pin (plastically-dominated contact).

5.9.4 PE-on-Metal versus Metal-on-Metal

Most work evaluating boundary lubrication considers metal-on-metal articulations. The differences between metal-on-metal and metal-on-PE articulations significantly impact the tribology of the articulations, however, and must be considered in the schematic understanding as presented in Figs. 5.9.1 and 5.9.2. One difference between the two articulating couples is that the PE surface deforms under load much

more than a metal surface. This deformation (Fig. 5.9.3) allows a good boundary lubricant to have a substantial effect on A_r. In metal-on-metal articulations, boundary lubricants may bear some load, and reduce wear, but typically they can only have a small effect on friction because they cannot greatly influence A_r.[56] For PE-on-metal articulation, the loading and geometry conditions determine the sum $A_r + A_l$. The relative portion of the surface in which there is direct PE-metal contact (*i.e.*, A_r) depends on the quality of the lubricant. A good boundary lubricant binds strongly to one surface and repels the other, even at a distance, thus making A_l large relative to A_r. A poor boundary lubricant does not perform this function, allowing A_r to remain large even with substantial deformation. In brief friction tests, this manifests itself in frictional differences; as is shown in the next chapter, a reduction in A_r brings about a reduction in volumetric wear rate.

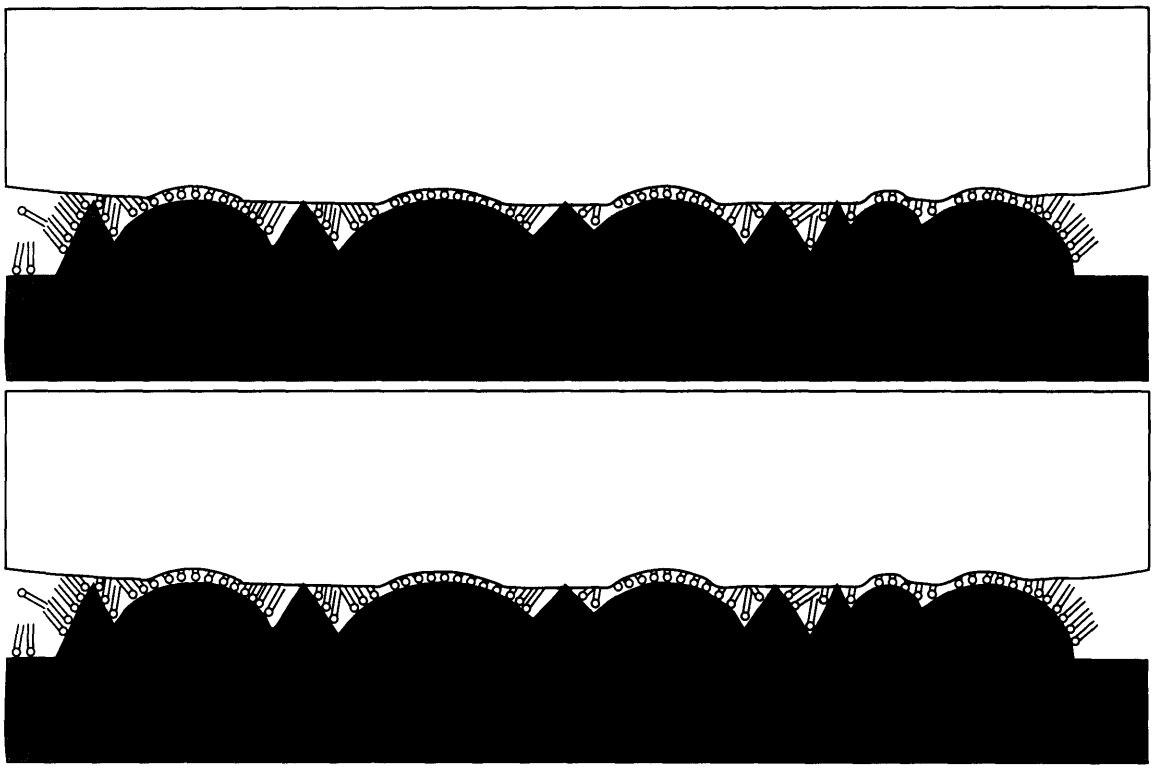

Fig. 5.9.3 Schematic of PE-on-metal articulation showing deformation of the PE surface Deformation enables increased boundary lubrication, increasing A_l (shaded above) and decreasing A_r (shaded below) relative to the metal-on-metal lubricated case and the PE-on-metal non-lubricated case. Note that this figure more accurately depicts the articulation of PE on metal than Fig. 5.9.2 does.

A second difference between metal-on-metal and metal-on-PE due to the increased compliance of PE is that a relatively small molecule can lubricate metal-on-PE, whereas large molecules are needed to lubricate metal-on-metal. In metal-on-metal, the best lubricants are phospholipids and fatty acids with long hydrocarbon chains capable of bearing load at a distance.[22,45] The length of these molecules may only be 5 to 10 nm, but they may form layered structures to bear loads over greater distances. Obviously a metal-on-metal couple would have to be quite smooth for molecules of this size to have much of an effect. Since PE is so much more compliant that metals, it is more possible for a relatively small molecule to have a protective effect.

Finally, in metal-on-metal articulations, both surfaces are hydrophilic. If boundary lubrication arises when a molecular species adsorbs to one surface and repels the other surface,[22] molecules with a hydrophilic portion and a hydrophobic portion (such as phospholipids and some proteins) are suited to metal-on-metal articulations, but not so with metal-on-PE articulations. In metal-on-PE, one surface is hydrophilic and the other hydrophobic. Thus, a molecule that is either hydrophilic or hydrophobic could adhere to one surface and repel the other. An amphiphilic molecule such as a phospholipid would not be necessary or even helpful.

Thus, there are several differences between the two articulations, leading to different types of molecule likely providing good lubrication. These differences are summarized below in Fig. 5.9.4.

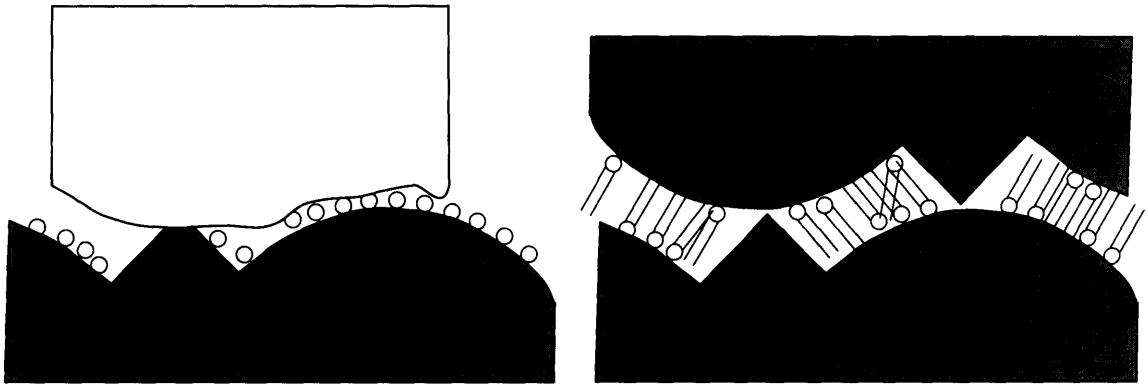

Fig 5.9.4 Comparison of metal-PE (left) to metal-metal (right) articulation Increased conformity and different chemical nature of surfaces leads to very different types of lubricant. Specifically, a relatively small globular hydrophilic (or hydrophobic) molecule can lubricate metal-PE, whereas a large amphiphilic is needed to lubricate metal-metal articulations. Although small molecules can function as lubricants for PE on metal, size is still an important and relevant parameter.

5.9.5 Ideal Friction Assay Conditions

Because the nature of the articulations is so different, the appropriate test to distinguish lubricants is likely different as well. An ideal geometry for evaluating lubricants under the PE-metal pair is one in which the boundary lubricant has a maximum opportunity to affect coefficient of friction. Therefore, $A_l + A_r$ should be fixed, but each component is maximally variable. Additionally, dead space should be minimal; that is, the area not bearing load, contributing only to noise, is minimized (*i.e.*, $A = A_r + A_l$ rather than $A > A_r + A_l$). One means to minimize dead space is to use a smooth metal counterface, minimizing the height of asperities and the valleys between them. Additionally, the use of a sphere-on-flat geometry and high load is consistent with an evaluation of lubricants. In this case, the sphere behaves more like a single asperity flattening out under load than a flat pin does. These conditions provide maximal opportunity for a good boundary lubricant to decrease A_r, distinguishing itself from a poor boundary lubricant. The present results are consistent with this discussion, since a sphere-on-flat under high load was ideal for distinguishing among lubricants. Despite the differences between surfaces, the ideal conditions in the PE-on-metal case are reminiscent of the use of a four-ball extreme pressure test to identify excellent boundary lubricants for metal-metal pairs.[45]

For a given metal-PE couple with a given topography, τ_w is independent of lubricant (though it may depend on other factors, such as load[57] or counterface roughness). Importantly, for a fixed geometry, when A_l increases (as through a more effective boundary lubricant), A_r decreases. Since A_r relates directly to friction force, friction force can estimate how effectively a boundary lubricant protects a surface.

5.9.6 Comparison of Boundary Lubricants for PE on Co-Cr on in Joint Fluid

Having established the utility of these friction experiments and having considered the likely characteristics of a good boundary lubricant for PE-on-metal articulation, it is now possible to assess the results of this chapter in greater depth. A key finding emerging from these experiments is the underappreciated boundary lubricating ability of water in PE on Co-Cr. Under the boundary lubrication model shown in Fig. 5.9.4, water can wet (adsorb to) the metal surface and repel the hydrophobic PE surface better than most other molecules. Thus, water could provide reasonable boundary lubrication for metal-on-PE. Since water is such a small molecule (~ 0.1 nm), its ability would be limited to very conforming articulations, as under high stress (as measured in this case). Other molecules may provide better boundary lubrication, particularly if they were large enough to act over gaps much larger than a water molecule. This boundary lubrication by water is not an entirely new concept,[56] though perhaps the fact that additives negatively affect its performance as a lubricant has not been previously shown.

Any molecule in the lubricant which adsorbs to Co-Cr and fails to repel PE as well as water does would increase the friction generated in articulation. The finding that albumin, γ-globulin, and HA increase friction is consistent with this understanding. These molecules could interfere with boundary lubrication by water in a number of ways. They may compete with water in adsorption to metal, perhaps even beginning to exclude water from parts of the surface. Alternatively, they may adhere to both metal and PE surfaces, increasing the interaction between the two. In any case, these molecules increase the friction of PE and metal relative to that of water lubrication. They do not increase friction nearly to that of dry sliding ($\mu_d \geq 0.2$).

Phospholipid, which would have a significant effect on the tribology of metal-metal pairs, has no effect on the articulation of PE-on-metal. In light of the above discussion, this finding is perhaps unsurprising, and needs no further explanation.

Finally, there must be one or more additional components in joint fluid that provide improved boundary lubrication above that of water in PE on Co-Cr. In bovine serum, the component(s) appear to be one or more proteins. In joint fluid, the component could be lubricin, which lubricates very highly conforming articulations such as cartilage-cartilage and latex-glass (cf. section 2.1.2). The component likely performs better than water because it is larger, and can bear a load over greater distance, though the true nature of its activity could be more complex, involving structured layers of lubricating molecules, for example. If the component of joint fluid is a protein, it would explain the paradoxical correlation between protein concentration and friction of PE on Co-Cr discussed in sections 5.6.2 and 5.6.5.

5.9.7 Co-Cr versus Ox-Zr

One possible explanation for the reduced friction of PE on Ox-Zr is a difference in the surface wettability.[40] Specifically, the ceramic surface of Ox-Zr adsorbs water better than the metal surface of Co-Cr. This rationale is consistent with our model of boundary lubrication by adsorbed water molecules, though it does not necessarily explain the fact that HA and protein negatively influence Co-Cr but not Ox-Zr. It may be that the affinity of Ox-Zr for water is sufficiently strong that other molecules are effectively excluded from the surface, and do not therefore have any effect.

Another explanation for the difference between the surfaces is decreased roughness in Ox-Zr. As mentioned briefly above, a smoother surface has fewer sharp asperities that are difficult to lubricate, thus leading to higher friction, and, as discussed in the next chapter, higher wear. If the Ox-Zr surface resists roughening better than Co-Cr, this difference alone could lead to the difference in friction. This explanation is consistent with the observation that the Ox-Zr surface appeared smoother than the Co-Cr surface despite the use of a consistent polishing protocol. This explanation is also consistent with the observation that, for individual tests, the oscillation of friction about μ_d was smaller in amplitude for PE on Ox-Zr than for PE on Co-Cr. This finding is not discussed above in the results, and is not quantified further, but could be pursued in later studies.

The roughness explanation is not consistent, however, with the fact that the negative effect of HA and protein on the tribology of PE on Co-Cr did not carry over to PE on Ox-Zr. This finding favors a chemical difference between the surfaces, such as wettability, as discussed above. A complete comparison of these two surfaces is beyond the scope of this thesis. These experiments do indicate, however, that PE on Ox-Zr performs better than PE on Co-Cr under adverse conditions (when lubricated by components of joint fluid that negatively affect tribology).

5.10 Limitations of the Current Study

The primary limitation of this study is the potential disconnect between friction and wear. Although the differences in friction measured in this study are relevant to the tribology of TJA, greater importance is tied to wear in TJA. There are both theoretical and empirical associations between friction and wear, and these can be carefully applied to the present results. Although the support for a connection between friction and wear in metal-on-PE is significant, the correlation between wear and friction is not universally accepted in all articulations. Some researchers have tried to show a strong correlation between friction and wear,[58] whereas others think friction has no relevance.[59] Section 5.9 begins to address the connection between friction and wear with conceptual and semi-quantitative discussion. The next chapter continues this discussion, relating the friction differences shown presently to clinically relevant differences in wear.

An additional and related limitation of this study is that differences among lubricants that were significant under high loads were less significant, or even absent, at low loads. A variety of loading conditions occur in the complex articulations that occur in replacement joints, likely spanning the entire range tested, but also including low stresses. From the present experiments, it is not clear whether the most important tribology occurs at these higher stresses, or at lower stresses. To an extent, this limitation

is also addressed in Chapters 6 and 7. Ultimately, the results of these experiments must be confirmed by a limited number of wear tests before conclusions can be applied to metal-on-PE arthroplasty.

A third limitation, associated with the measurement apparatus, was the saturation of measurement at high forces. It was evident that higher loads and stresses led to more significant differences between groups. Load could not be increased further, however, because the true frictional force would have been truncated. Even at the 589 g load, several values of μ_s were truncated. Fortunately, with two measures of friction (dynamic and static), useful comparisons could still be made. To exemplify this limitation, it would have been desirable to obtain friction measurements for the non-lubricated (dry articulation) case to compare with other results. Measurements were made under 59.9 g load using a 6.4 mm diameter flat pin ($n = 5$, $\mu_s = 0.53 \pm 0.12$, $\mu_d = 0.31 \pm 0.09$). These results cannot be compared to the majority of results presented, since they were obtained using a different type of pin and higher loads. Measurements could not be made under high load and with a small spherical pin because these measurements would have been truncated at $\mu \sim 0.19$. Thus, even dynamic measurements would likely have been cut off.

If truncation were not an issue, static measurements might be of more interest than dynamic, since start-stop motion dominates and steady motion occurs only a small fraction of the time. On the other hand, static friction represents a single measurement, and is thus more subject to noise than dynamic friction, which is averaged over 40 seconds. So even without truncation, dynamic friction may have been more practical than static. Chapter 7 briefly describes a more sophisticated device for friction measurement that avoids the truncation problem; this apparatus could be useful in future studies.

Finally, the rapidness of the friction assay could mask clinically relevant time-dependent effects. For example, a potential time-dependent effect of proteins is suggested by the data. Examining closely the results of protein lubrication, protein affected μ_s only at high concentration, but increased μ_d at both low and high concentration. This finding could be related to time-dependent protein adsorption to Co-Cr. A possible interpretation of these results is that surface adsorption of protein depends on concentration, occurring within seconds under high concentration and requiring closer to a minute under low physiological concentration (thus generating different responses in immediate and delayed tests).

To investigate the effect of time on this tribological assay, two samples of PE on Co-Cr were tested with bovine serum as lubricant in the following fashion. The assay was performed once immediately after application of bovine serum. After friction was measured, the PE surface was cleaned and removed from the metal. The Co-Cr surface, bathed in bovine serum, was covered with plastic wrap to prevent interactions with the atmosphere, (*e.g.*, as evaporation). Three hours later, the plastic wrap was removed, and the test repeated. The results from the consecutive tests were compared: static friction was identical immediately and after a delay ($\mu_s = 0.14 \pm 0.01$ versus 0.14 ± 0.02), but dynamic friction decreased significantly after three hours ($\mu_d = 0.076 \pm 0.007$ versus 0.062 ± 0.005, $p = 0.035$). This decrease suggests some kinetic adsorption of bovine serum components to the Co-Cr surface, leading to enhanced boundary lubrication after a

three hour wait. On the other hand, the conflicting results for static and dynamic friction are hard to interpret.

It should be noted that the bovine serum standards run concurrently with these tests measured significantly higher coefficient of friction than previous standards run months earlier in conjunction with the majority of experiments. Because it was difficult to explain the change in standard values outside a difference in the PE pins, comparisons are only made between tests performed in this series using the same PE pin. Obviously, the results of two tests are insufficient to draw meaningful conclusions, particularly with the unresolved question of abnormally high standard measurements. Nonetheless, these results suggest time is an important factor to consider, at least within the first three hours. It is difficult to interpret which of these times might be more appropriate to use. TJA surfaces are exposed to joint fluid throughout their lifetime, so the effect of lubricant after long times may be most appropriate. On the other hand, since in the areas of highest wear, lubricant is sheared from the implant surface with each step, tribological measurements immediately after exposure to lubricant may be the most meaningful.

Relating to the present results, it is unlikely that these time-dependent effects would change the relative ranking of lubricants. Therefore, the essential results of this chapter are not significantly altered by this finding. Certainly, this is a topic of interest, however, and would be worth examining in the future. In this apparatus, waiting a longer time could introduce non-physiological effects, such as protein aggregation and precipitation. Thus, more complex means may have to be devised to examine longer time-dependent effects.

5.11 Conclusions and Relevance

By choosing a relatively high load and small radius spherical pin, it was possible to distinguish among lubricants in the articulation of PE-on-metal using a friction assay. The frictional differences between water and bovine serum were consistent with the typical ranking of these two lubricants in wear simulator studies (*e.g.*, McKellop *et al.*, 1978;[1] Derbyshire *et al.*, 1994;[60] Wang *et al.*, 1996;[61] and Besong *et al.*, 1999[62]). Additionally, wear rates under serum-lubricated conditions are similar to typical *in vivo* wear rates. This result is confirmed by the similar coefficients of friction measured for bovine serum and some joint fluid samples. Furthermore, some replacement joint implants wear at significantly higher rates than the average. This clinical finding may be explained by poor tribology of PE on Co-Cr when lubricated by certain joint fluid samples, including those from failed prostheses. Although it is not possible to define a quantitative relationship between friction and wear, differences in friction can be used to distinguish and to rank lubricants.

HA and protein were found to have significant effects on the tribology of PE on Co-Cr when compared to saline lubrication, but none of the components measured affected friction of PE on Ox-Zr except to reduce variability. Finally, clear variability was shown in the lubricity of joint fluid in this articulating pair. Given the variability in PE on Co-Cr tribology under joint fluid lubrication, highly variable clinical wear rates are expected. Although the source of this lubricity was not uncovered, HA, albumin, γ-globulin, and phospholipid are excluded as candidates. Other components of joint fluid, such as other proteins, are likely candidates. The reduced friction and reduced variability

under PE on Ox-Zr articulation suggests that these implants are less likely to encounter the highly variable PE wear rates that lead to prosthesis failure. It is still not clear what component of joint fluid improves upon the boundary lubrication of PE on Co-Cr over distilled water.

These findings all relate to the clinical outcome of TJA. Although PE wear particle generation is the fundamental problem, friction in this assay marks for poor boundary lubrication leading to PE wear generation *in vivo*, as discussed further in Chapter 6. Thus, this assay is highly relevant to clinical issues of joint fluid and TJA.

Considering all articulating couples for joint prostheses, the fact that different surfaces respond differently to serum, proteins, HA, phospholipids, etc. suggests that it will not be easy to "simulate" joint fluid *in vitro*. In PE on Co-Cr, it is found that a component other than albumin, γ-globulin, HA, or phospholipid substantially determines the tribology, though these components have some impact. In another couple, however, these or other components could be of paramount importance, so it is not clear how one would "simulate" joint fluid for a wear test on a new articulating couple. Until the effect of each component on a particular couple is better understood, it is difficult to justify leaving out *any* component of joint fluid in a "synthetic joint fluid" for laboratory wear tests.

5.12 References

This work is currently under consideration for publication in condensed form.

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CHAPTER 6

LOAD, AREA, AND POLYETHYLENE WEAR

Moving now from the study of the effect of lubricant in replacement joint articulations, this study discusses the effect of other parameters in laboratory studies. This study completes the thesis in that it evaluates wear, which is a more direct measure than friction of the performance of an articulating couple in this application. Furthermore, it ties together friction and wear, and provides an opportunity to apply the conceptual framework developed in Chapter 5 to wear in PE on Co-Cr.

The effects of contact area and contact stress on the wear of PE articulating with a polished surface of Co-Cr alloy were evaluated using a pin-on-disk apparatus implementing bi-directional movement. Within a relevant range of contact stress, volumetric wear rate increased with increasing contact area. Volumetric wear was found to be independent of normal load within this range. Small but statistically significant changes on coefficient of friction were associated with large differences in wear rate. These findings are considered within the framework of a conceptual understanding of PE-on-metal articulations developed in the previous chapter.

6.1 Introduction and Objectives

There continues to be considerable uncertainty regarding the principal determinants of wear of PE in total joint arthroplasty articulations. Moreover, while extensive effort has been made to employ pin-on-flat articulation in the laboratory to simulate tribology extant in hip and knee arthroplasty, there are still questions regarding parameter specification[1-6] in such apparatus. Although certain features such as bi-directional articulation[7,8] have been well-established as important in laboratory tests, the roles of other parameters, such as contact area, load, and nominal contact stress, have not yet been established.

6.1.1 Wear Rate Dependent on Normal Load?

For many non-medical wear couples, volumetric wear (V) has been found to be proportional to normal load (W) and sliding distance (l), and dependent upon factors related to the articulating surfaces, including hardness and real contact area.[9] When considering the wear of polymers, factors relating to the articulating surfaces have typically been combined into a "wear factor" (k), such that $V = l \times W \times k$ *(Equation 2.4.1).*[10] This type of analysis has typically been applied to metal-on-polymer arthroplasty (*e.g.*, Wang[8]). Radiographic and prosthesis retrieval studies produce data suggesting that k is on the order of 2×10^{-6} mm^3/Nm.[11,12] Consequently, pin-on-flat studies are routinely judged against this clinical wear factor. Using clinical wear factor to assess the validity of pin-on-flat wear tests is only appropriate if wear depends linearly on normal load, and if other parameters, such as contact area and contact stress, are either irrelevant or well-matched *in vitro*.

6.1.2 Wear Rate Dependent on Contact Stress?

Several studies have suggested that the clinical wear factor inadequately reflects the influence that design factors have on wear in metal-on-polymer arthroplasty. Some of the early laboratory studies showed that, at low contact stress, wear rates were very low, and at high contact stress, wear was exponentially related to pressure. Rostoker *et al.* reported that this transformation took place at a critical stress of 7 MPa, but his tests were unidirectional, and lubricated by distilled water.[13] Rose *et al.*, also using unidirectional motion, reported a pressure-velocity product exceeding a critical value, above which wear depends exponentially on contact pressure.[14] These studies suggested that the essential loading parameter determining PE wear is contact stress.

6.1.3 Wear Rate Dependent on Contact Area?

By contrast, a number of clinical studies have shown an increase in volumetric wear in hips with larger acetabular cups. A summary of twenty-one studies of 22, 26, 28, and 32 mm acetabular cups[15] reveals a linear relationship between volumetric wear rate and acetabular cup surface area. Other clinical retrieval studies[11,16] as well as finite element models have related an increase in volumetric wear to femoral head size. These results suggest that volumetric wear in this articulation increases with contact area, though the increased sliding distance of larger heads likely contributes to this finding.

When referring to POF tests, a few terms must be defined. The nominal contact area used in this chapter is the same as A_a as defined in Chapter 5. The nominal or apparent contact stress is the normal load divided by A_a. These parameters are easier to define and measure than the actual applied stresses and areas of contact, which, as discussed in Chapter 5 and presently, depend on surface characteristics such as roughness.

A relationship between volumetric wear and contact area is suggested by recent *in vitro* data, as well. For example, Sathasivam *et al.* showed an increase in wear rate with nominal contact area over the range 50 to 110 mm^2 in a bi-directional POF test, though still larger nominal contact areas resulted in negligible wear.[2] The authors offered lubricant starvation as an explanation for the apparent contradiction between their results and their expectation that wear rate would increase with contact pressure. In another study, Saikko and Ahlroos compared 7.1 and 62 mm^2 polyethylene wear faces in a multidirectional device, finding a higher wear rate with increased nominal contact area. They did not report the wear rate with the smaller surface, however, since it did not reveal a "wear factor" of the same magnitude as is found clinically.[5] Finally, a hip simulator study conducted by Wang *et al.* in 2001 showed an inverse relationship between nominal contact stress and wear rate under constant load.[17] This inverse relationship under constant load suggests a direct relationship between nominal contact area and wear rate.

6.1.4 Specific Aims

The objective of the present study was to determine how PE wear rate depends on load, contact area, and nominal stress in a pin-on-flat test within a range of stresses encountered *in vivo*. This is achieved by bi-directional wear tests using PE pins of various diameters on a Co-Cr counterface using various normal loads, so as to vary nominal contact stress and nominal contact area independently. These results are considered in conjunction with those reported by others to more usefully develop the concept of a "wear factor" to better compare *in vitro* results of pin-on-flat tests. An illustrative model, introduced in Chapter 5, is expanded to explain these findings. Other parameters, such as lubricant replenishment protocol, test duration, and measurement interval, were also investigated with respect to their effects on volumetric wear. Additionally, the friction of PE and Co-Cr was measured under each articulating condition. This enabled a correlation between friction and wear in this couple.

6.2 Materials and Methods

6.2.1 Experimental Design

Volume loss was measured in the bi-directional articulation of PE on Co-Cr using selected nominal contact areas and normal loads. Wear tests were performed on a six station OrthoPOD apparatus (AMTI, Watertown, MA). Static loads of 111 or 223 N were applied to pins with diameter 4.8, 6.4, or 9.5 mm (with corresponding nominal contact area 18, 32, or 71 mm^2, respectively). This selection of loads and areas provided nominal contact stresses ranging from 3.1 to 7.0 MPa. Nominal contact stress is defined as the ratio of normal load to the area over which the load can act. In each experiment, three or six samples from each group were tested for 0.5 million cycles (Mcycles) or 1.5

Mcycles. Table 6.2.1 shows a matrix of the load and area combinations that have been tested. These combinations enabled a direct comparison of the effects of nominal contact area and nominal contact stress on the wear rate of PE in this articulation.

Table 6.2.1 Wear test experimental matrix This table shows the various parameters for each experimental group. For two groups, each lubricant replenishment protocol was used for three pins. This matrix allowed for comparison between groups of identical nominal contact area (2 & 4), identical normal load (2 & 3), and similar nominal contact stress (1 & 2, 3 & 4). Groups 2 and 3 allowed for comparison of the two lubrication replenishment protocols. *Lubricant was 40% bovine serum, 20% HA solution, and 40% distilled water

Group (Sample Size)	*Diameter (mm)*	*Area (mm^2)*	*Normal Load (N)*	*Contact Stress (MPa)*	*Lubricant Protocol*	*Duration (Mcycles)*
Pilot (n = 3)	9.5	71.2	111	1.6	A*	0.5
1 (n = 3)	4.8	17.8	111	6.3	A	0.5
2 (n = 6)	6.4	31.7	223	7.0	A,B	1.5
3 (n = 6)	9.5	71.2	223	3.1	A,B	1.5
4 (n = 6)	6.4	31.7	111	3.5	B	1.5

6.2.2 Pins and Disks

The PE pins, provided by Smith & Nephew (Memphis, TN), were machined to implant grade smoothness from accepted rod stock of PE (GUR 1150; Westlake Plastics, Lenni, PA) as per ASTM F648 (type 2). The pins were 19 mm in length. The mean density of the pins was 0.925 mg/mm^3. The pins were used as received and were not sterilized. After the tests, pins were examined both grossly and microscopically (SZ-PT optical microscope, Olympus, Japan).

The Co-Cr disks serving as metal counterfaces were 35 mm in diameter by 6.4 mm thick. They were drawn from accepted bar stock (Smith & Nephew; Memphis, TN), polished according to the procedure used for femoral knee components to 25 to 50 nm R_a, as per ASTM F1537.

The average roughness of three pins, randomly selected, was measured before and after wear testing using a Tencor P10 surface profilometer (Santa Clara, CA) with 2 μm stylus. Three disks were also measured after wear testing (for each disk, $R_a < 50$ nm before the start of the wear test). For profilometry, each pin (or disk) was oriented at random on the profilometer stage, and at least 5 mm of the surface was traced by the stylus at 20 μm/s. Average roughness, R_a, was used as a measure of the surface topology. The measurement was repeated twice after reorienting the specimen such that, in total, three random diameters were profiled from each pin and disk.

6.2.3 Experimental Parameters

Each wear test was comprised of six pins, each subject to a fixed load, moving cyclically on its own metal disk. Movement of the pin relative to the disk was in a square pattern, 10 mm on a side. The length of the side was chosen to match the long dimension of the wear path reported for the path traced by the femoral head on the acetabular cup.[18] The square pattern was chosen to prevent the preferential alignment of PE in the principal direction of sliding,[8] thus maximizing the generation of wear particles per unit sliding

distance. Each wear test was run at a rate of one cycle per second, with a mean speed of 40 mm/s. This rate was chosen to maximize the total amount of wear generated in a given time period without departing from *in vivo* wear mechanisms. The square pattern was formed by tracing 20 points forming the perimeter of a square. The distance between each pair of points was covered in 50 ms, thus ensuring relatively constant speed. For further discussion of the means for generating the square pattern using rotation about two independent axes, see Appendix N.

Each disk was placed in an individual well containing 15 ml of newborn bovine calf serum (lot number 1023609, catalog number 16170; Invitrogen Corporation, Carlsbad, California), diluted to 40% to contain 29 mg/ml protein. Surrounding the six wells was a water bath maintained at 37°C. A pilot study employed a modified lubricant including the addition of an HA solution at concentration 20%. The solution was a mix of viscosupplements, as described in section 3.2.

Before the start of the test, the pins were weighed on a balance with resolution to 0.1 mg. At regular intervals during the wear tests, the pins were removed from the experimental apparatus, wiped clean of adherent lubricant, and weighed. No drying protocol was used prior to weighing or after the tests to reduce the effects of absorbed water. Mass loss was converted to volume loss using the density of the pins. Each time the lubricant was to be replaced, all six disks and wells were cleaned thoroughly in detergent soap, rinsed in tap water, then rinsed again in distilled, deionized water before being refilled with 15 ml calf serum. A new set of pins were used for each wear test.

The series of experiments were originally designed such that pins were weighed at intervals of 0.5 Mcycles (~ 6 days). Every 0.25 Mcycles (69 hours), distilled water was added to the lubricant to account for evaporation. This lubricant replenishment protocol, which is titled protocol A, was based in part on previous pin-on-flat experiments suggesting the need to supplement evaporated lubricant with distilled water.[2] After running Group 1 and a portion of Groups 2 and 3 using this protocol, it became evident that adding distilled water was not necessary – evaporation of the lubricant over the course of 0.3 Mcycles was insufficient to warrant replenishment. Consequently, a second protocol was employed. In the second protocol (B), pins were weighed at intervals varying from 0.11 to 0.36 Mcycles (30 to 100 hours), and the lubricant was not disturbed in the interim. Lubricant replenishment protocol B was employed on the second three samples of Groups 2 and 3 after 0.5 Mcycles. Due to the improved repeatability using protocol B (as defined by reduced coefficient of variation and greater linearity), this protocol was used in all future tests. When possible, the two lubricant replenishment protocols were compared.

Preliminary tests were performed to determine whether absorption of water would affect the mass of PE during the course of the tests on this experimental apparatus. Five 9.5 mm pins were maintained under non-articulating load of 70.7 N in bovine serum for the temporal equivalent of 1.6 Mcycles. These were performed using lubricant protocol A.

6.2.4 Friction Measurements

In addition, efforts were made to periodically measure friction of PE on Co-Cr using the POF apparatus. Every 50,000 or 100,000 cycles, the OrthoPOD software was

programmed to briefly interrupt the wear test and raise all of the pins. During the interruption, each pin in succession was run for a small number of cycles in a reciprocating 10 mm line under the same normal load as the bulk of the test. These tests were also run at 1 Hz, for a mean speed of 20 mm/s. Normal and transverse forces were measured during this reciprocation, such that frictional force and coefficient of friction could be calculated. Measurements were taken every 20 ms, for a total of 50 measurements in one cycle. After each POF couple had been evaluated in this way, the bidirectional wear test resumed.

6.2.5 Statistical Methods

Volume loss was determined from mass loss and presented as a function of number of cycles (or, equivalently, distance traveled) by linear regression. Results were calculated with and without the line passing through the origin. The slope of this line, the wear rate, has been reported in units of cubic millimeters per Mcycle and cubic millimeters per meter of sliding distance. In figures, only the former units are given.

For 0.5 the Mcycle test, a sample size of $n = 3$ was used. Using a two-tailed unpaired Student's t-test, this sample size is sufficient to determine a 24% difference in wear rate (with $\alpha = 0.05$ and $\beta = 0.1$) between two groups with a 10% coefficient of variation. For 1.5 Mcycle tests, a sample size of $n = 6$ was used. This sample size is sufficient to determine a 17% difference in wear rate (with $\alpha = 0.05$ and $\beta = 0.1$) between two groups with a 10% coefficient of variation.

6.3 Results

6.3.1 Pin and Disk Morphologies

Before the tests, the PE pins had a mean R_a of 18 nm. Under magnification, machine marks were visible on the surface of all PE pins. The machine marks were concentric, 5 to 20 μm in depth, and 80 to 250 μm wide; these machine marks were too large to greatly affect R_a. In all cases, the PE surfaces appeared polished or burnished after 0.1 to 0.4 Mcycles. After 0.3 Mcycles, pimples 0.2 to 0.5 mm in length by 0.1 to 0.2 mm in width were visible on many of the PE wear surfaces. These were visible on all PE wear surfaces by 0.5 Mcycles. The appearance of pimples continued throughout the tests until reaching an approximate linear density of one pimple per 0.5 mm. The mean R_a of the PE pins after the test was 2.6 nm, reflecting polishing by the metal disks and the diminution of machine marks. Under microscopy, irregular features of size 20 μm high by 300 μm wide existed on the pins.

Before wear tests, the Co-Cr disks were smooth and mirror-like, and each disk tested fit within the range prescribed in ASTM F1537. On some disks, the articulating surface became increasingly scratched over the course of the experiments. Despite these scratches, R_a of the disks measured after the tests was 3.6, 3.8, and 3.9 nm.

6.3.2 Wear Measurements

In the first 8 days (0.7 Mcycles), the loaded control pins gained 0.2 ± 0.2 mg (mean ± standard deviation). There was little change over the remainder of the test, with final increase from the start of the test being 0.3 ± 0.2 mg. These mass differences were

on the order of the resolution of the balance, which likely explains the high coefficient of variation. For pins of this size, this mass gain was small (< 5%) relative to the overall mass loss of the worn pins. Since the normal load was not identical to that used in the wear tests, and since several different sized pins were used for wear tests, the data were not adjusted by this absorptive mass gain.

In wear tests, gross changes in the lubricant were apparent in the interval between measurements. Before the experiments, the serum had a brown-red tint, and was essentially transparent. By about 0.1 Mcycles, however, the serum began to turn a creamy yellow color of increasing opacity. White aggregates of various irregular shapes on the order of 5 mm in length began to appear suspended in the serum and adherent to the Co-Cr disk.

The lubricant in the loaded soak control pins did not undergo these changes, however. Over time, the lubricant level dropped and the serum turned a darker shade of brown-red, both evidence of evaporation. A film did adhere to the disk surface in each of the controls. When the loaded pin was removed from the disk, the circle of contact between the disk and pin contained no adherent film.

6.3.3 Friction Measurements

The OrthoPOD software was designed to employ one articulation pattern per test. Employing a second pattern met with mixed success. Specifically, this second pattern did not happen much of the time. The software simply ignored this portion of the test. This poor reliability likely did not affect wear measurements, since the addition or subtraction of ten unidirectional measurements would not greatly affect the wear of PE over 0.1 Mcycles. Due to the poor reliability of measurement, however, these data were not compiled for publication. Upon the completion of the friction experiments of Chapter 5, however, it became more pressing to determine a relationship between friction and wear in this couple. Thus, the data were revisited.

As a sample friction measurement, I show data from a preliminary experiment, in which three 9.5 mm diameter pins and three 4.8 mm diameter pins were run under 111 N normal load for 0.5 Mcycles. This test was conducted using lubricant protocol A. The three 9.5 mm diameter pins were lubricated by calf serum plus 10% by volume HA. The HA added was in liquid (injectable) form, and was of unknown concentration and molecular weight (hence a pilot study). The other three pins were from Group 1A (lubricated by bovine serum).

Fig. 6.3.1 shows a trace of coefficient of friction versus time for the pilot group (HA added) at 50,000 cycles. This trace is typical of these friction measurements. Although the measurements were affected by direction changes each half second, it is difficult to pinpoint the direction change. Since data were occasionally erratic surrounding the direction change (*i.e.*, $\mu > 1$ or $\mu < 0$), and since these erratic periods were not easily removed from the analysis, the median friction measurement was employed (rather than an average measurement that would be greatly affected by outlying points). For statistical analysis, each median friction value was treated as a single data point. The median friction value measured in this case is given in Fig. 6.3.1.

Median friction values for each pin were compiled to obtain an average coefficient of friction at each time point. These averages were examined graphically

versus number of cycles to evaluate changes in friction over the course of the experiment. When changes were not observed, the data were averaged to obtain a mean coefficient of friction for each test condition. First, values were compared within an individual experiment (that is, two different pin types under the same load and experimental conditions). Then, when necessary, comparisons were made across experiments. Further discussion of coefficient of friction in other groups is given when appropriate.

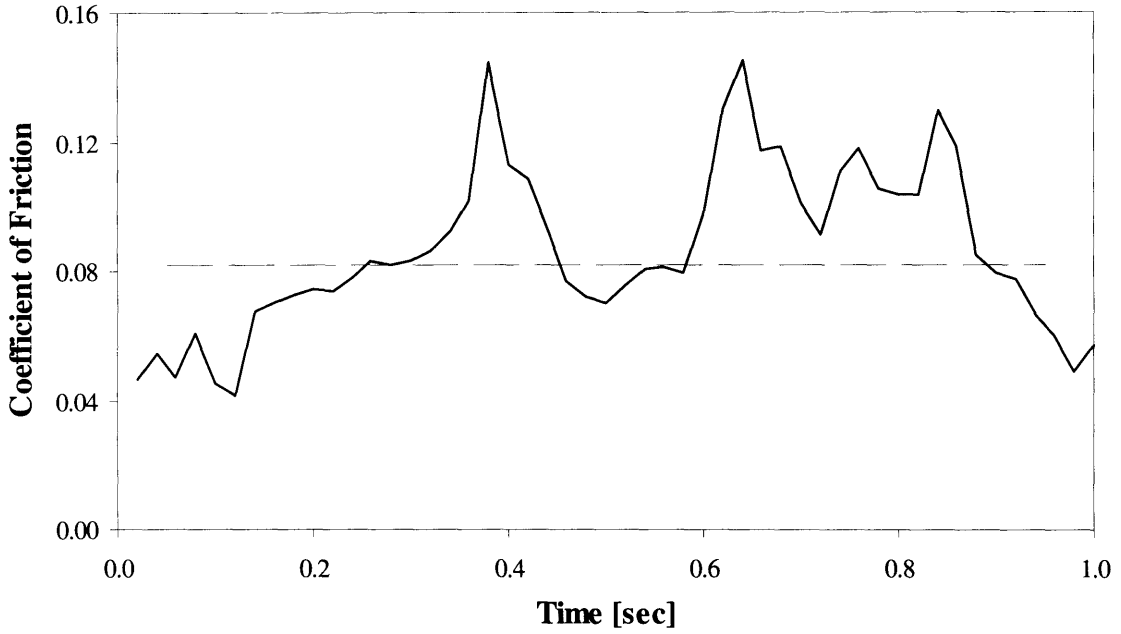

Fig. 6.3.1 Sample trace of coefficient of friction versus time This trace was taken from the first pin of the pilot study after 50,000 cycles. Experimental conditions were: 111 N normal load, 9.5 mm diameter pin, 40% v/v bovine serum plus 20% HA as lubricant. Note that the erratic peaks and valleys do not quite correspond to directional changes at 0.5 sec and 1.0 sec. The dotted line indicates median coefficient of friction.

As an example, the measurements obtained from the pilot study are given below in Fig. 6.3.2. In all cases, the coefficient of friction was higher for the larger pins than for the smaller. After 250,000 cycles, the calf serum used as a base lubricant for these experiments was replaced with a new batch. After this time, the coefficient of friction in both groups increased (Group 1: increased from 0.050 ± 0.015 to 0.13 ± 0.03; pilot group: increased from 0.089 ± 0.021 to 0.19 ± 0.03). Differences between the two sets of pins and between the first and last 0.25 Mcycles were both highly statistically significant ($p < 0.0001$). Considering the friction for both groups averaged over all measurement points, the difference was still highly statistically significant ($p < 0.0001$). After this test, friction was measured less often (every 0.1 Mcycles). The mean friction averaged over all time points for all groups is given in Table 6.3.1.

It is difficult to draw conclusions from the friction measurements in the pilot study because both lubricant and pin size differed between the two groups. Since the pilot study was ended after this first group, little significance can be drawn from its results besides the connection between coefficient of friction and wear. Furthermore, this experiment, being the first in the series, was subject to a number of disturbances, including drying of the lubricant bath and changes in bovine serum batch. Thus, the

results of these two groups are best compared only to each other, rather than to other groups.

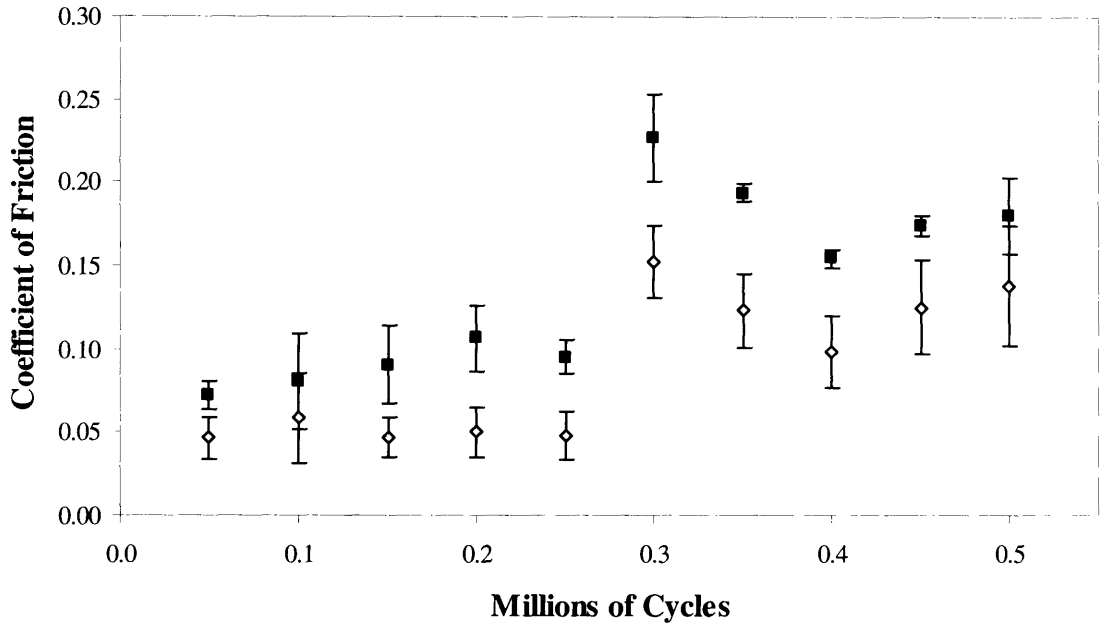

Fig. 6.3.2 Friction versus number of cycles in the pilot experiment Dark squares indicate the pilot group; light diamonds indicate Group 1. After 250,000 cycles, a different batch of bovine serum was used as lubricant. Thus, in the text, comparisons are made considering these two groups separately.

6.3.4 Lubricant Replenishment Protocol

For all groups, volume loss increased linearly with number of cycles or sliding distance as expected (Fig. 6.3.3). When the last Mcycle of three pins of groups 2 and 3 were performed using lubricant replenishment protocol B (Fig. 6.3.4), wear rate more than doubled under protocol B relative to protocol A. This difference was statistically significant by Student's *t*-test ($p < 0.001$). The wear rate was calculated as the slope of line that best fit the data. Furthermore, protocol B exhibited a greater degree of linearity relative to protocol A, as evident from linear regression analysis. Each experiment using protocol B yielded a coefficient of determination, R^2, of 0.99 or greater both for a line through the origin and for a line not tied to the origin. This contrasted with wear curves generated using protocol A, which had coefficients of determination as low as 0.68 using linear regression through the origin and 0.74 not using the origin. Moreover, the data generated using protocol B had a coefficient of variation of 11% versus 36% using protocol A, comparing each group's final data point, which should represent the highest mean and therefore lowest coefficient of variation. These results were typical of those obtained from groups in which the two protocols could be compared. Due to the lower variability in the test data using this second protocol, protocol B was found preferable, and was used for Group 4.

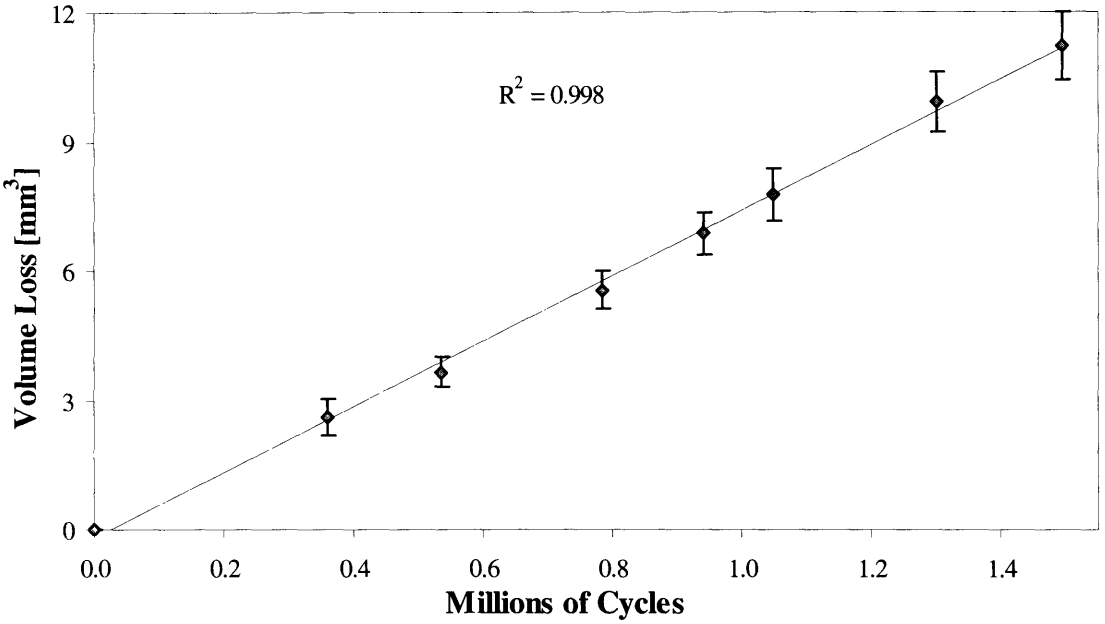

Fig. 6.3.3 Relationship between volume lost and number of cycles for Group 4B Line and coefficient of determination shown are for best fit line. The y-intercept of this line was -0.21 mm^2 (-0.20 mg), approximately the amount of water absorbed by the loaded control pins. Bars represent standard deviation.

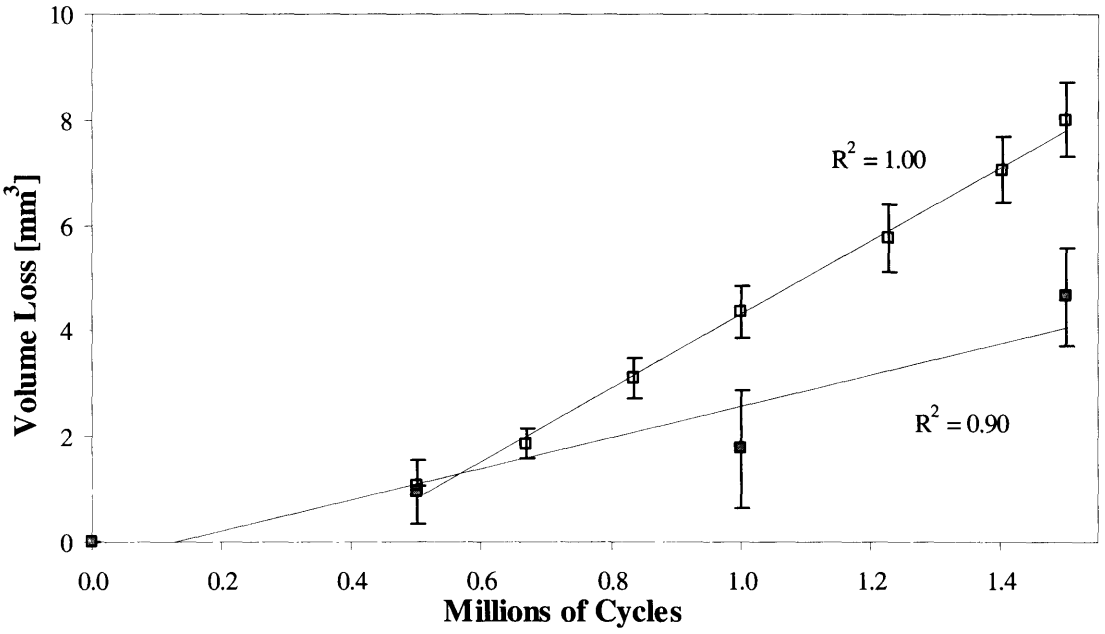

Fig. 6.3.4 Experiments run with 223 N normal load and pins with nominal contact area 72 mm^2 Closed squares represent experiments using lubricant replenishment protocol A for 1.5 Mcycles. Open squares represent experiments using lubricant replenishment protocol A through 0.5 Mcycles, and lubricant replenishment protocol B through the remaining 1.0 Mcycles. In each case, the lubricant was replaced at each measurement. Linear regression analyses and coefficient of determination are shown. Bars represent standard deviation, with $n = 3$ in each case. For the open squares, standard deviation represents only the variability arising during the period in which protocol B was used.

6.3.5 Experimental Protocol

Using lubricant replenishment protocol B, the interval between measurements varied in the range 0.11 to 0.36 Mcycles. This was done to enable continuous wear testing while taking measurements on a regular schedule. Furthermore, the utility of the test depends on the number of measurements, so it was desirable to make measurements as often as possible with minimal disruption of lubrication and wear mechanisms. Through the range of 0.11 Mcycles to 0.36 Mcycles, the wear in a given interval was proportional to the number of cycles – *i.e.*, wear per cycle was independent of the duration of the interval between measurements (Fig. 6.3.5). These results indicate that wear proceeded at the same rate regardless of the number of cycles between measurements, justifying the range of intervals chosen using this lubricant protocol.

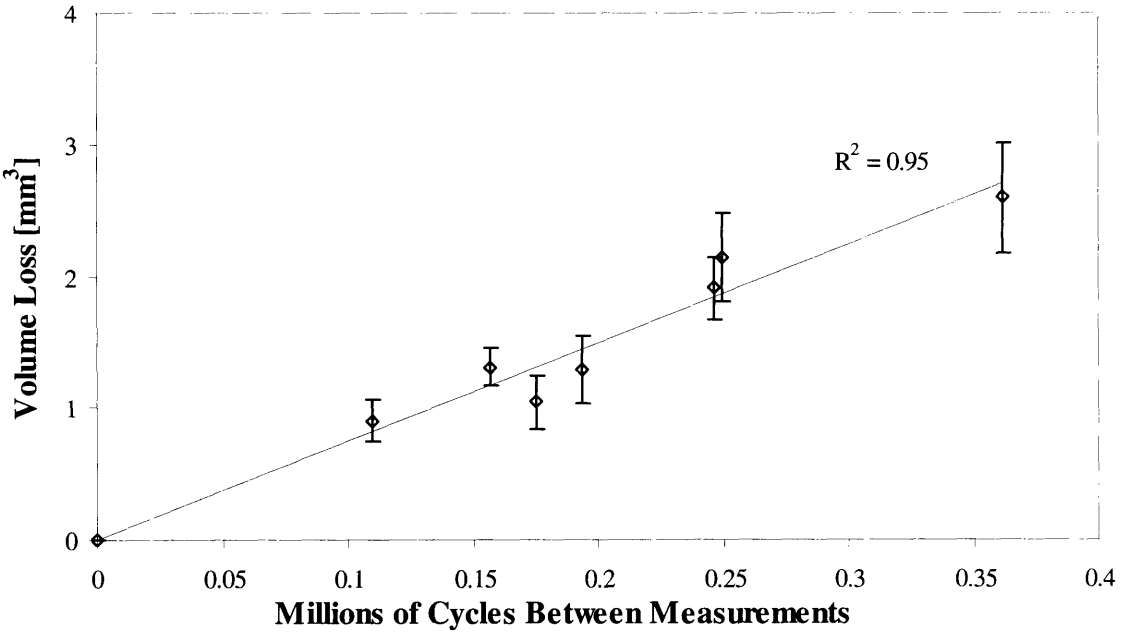

Fig. 6.3.5 Comparison of interval between measurements to change in volume Error bars represent standard deviation, with $n = 6$. The line shown is a best fit line through the origin. These data are from Group 4: 223 N normal load and 6.4 mm diameter pins, using lubricant replenishment protocol B.

Using lubricant protocol B, the linear relationship between volume loss and sliding distance extended from the first measurement, rather than after a period of non-linear wear, though the best fit line intercepted the y-axis at a *gain* of 0.3 ± 0.2 mg (0.3 ± 0.2 mm^3). This effect is likely accounted for by fluid uptake, as shown by the loaded soak control pins. Due to the goodness of fit, all data are presented as wear rates; that is, volumetric wear per cycle (or per meter sliding distance).

In tests in which 1.5 Mcycles were performed, linear regression was performed on the first 0.5 Mcycles as well as the complete 1.5 Mcycles, to evaluate the utility of the shorter wear experiments performed on Group 1A. Comparisons were made for Groups 2A, 3A, and 4B, comparing the values obtained in the first 0.5 Mcycles versus the data obtained from the duration of the test. In each case, the mean wear rate obtained in the first part of the test was less than that obtained from the full test. This difference was statistically significant in the case of Group 4 by Student's t-test ($p = 0.03$, $\beta = 0.61$).

This difference was not statistically significant in the case of Groups 2A and 3A, though the difference between the means was larger in these groups.

6.3.6 Experimental Parameters

The test groups (Table 6.3.1) allowed for three types of direct comparisons: groups with different contact area and the same normal load; groups with the same nominal contact stress, but different contact area and normal load; and groups with the same contact area and different normal load. In these comparisons, the effect of contact stress has not been considered as a separate parameter. Since the three parameters contact area, load, and nominal contact stress are interdependent, a relationship between wear rate any two parameters defines the relationship with the third parameter.

Table 6.3.1 Nominal contact area and load results matrix This table shows the wear rates and mean coefficients of friction obtained for each experimental group. Results are given as mean ± standard deviation. Area is given in mm^2. Wear rates are given in mm^3/Mcycle. "Initial wear" indicates wear rate over the first 0.5 Mcycles (*i.e.*, the volume change in the first 0.5 Mcycles divided by 0.5 Mcycles). This value is not given for Groups 2B and 3B because the first 0.5 Mcycles of these tests employed protocol A. Wear rates are not given for the pilot group and Group 1A because the experiment was only run for 0.5 Mcycles. μ = mean coefficient of friction averaged over all measurements made.

Group	*Area*	*Load*	*Contract Stress*	μ	*Initial Wear*	*Wear Rate*
Pilot (A)	71.3	111 N	1.6 MPa	0.137 ± 0.055	6.5 ± 0.8	N/A
1A	17.8	111 N	6.3 MPa	0.089 ± 0.046	3.2 ± 0.3	N/A
2A	31.7	223 N	7.0 MPa	0.034 ± 0.009	1.9 ± 2.1	3.0 ± 1.1
2B	31.7	223 N	7.0 MPa	0.037 ± 0.006	N/A	7.0 ± 0.7
3A	71.3	223 N	3.1 MPa	0.041 ± 0.007	5.4 ± 2.8	8.1 ± 2.9
3B	71.3	223 N	3.1 MPa	0.057 ± 0.016	N/A	16.0 ± 2.1
4B	31.7	111 N	3.5 MPa	0.043 ± 0.016	6.8 ± 0.7	7.6 ± 0.5

Effect of Contact Area Independent of Normal Load

The effect of increasing area (a 2.3-fold increase in area) on wear was evaluated in two experimental groups using the same applied load of 223 N with both lubricant replenishment protocols (comparing Groups 2A and 3A, and Groups 2B and 3B; Table 6.3.1). There was a greater than twofold increase in wear rate associated with larger nominal contact area for the two lubricant replenishment protocols. Using analysis of covariance, there were statistically significant effects of contact area ($p = 0.0002$) as well as lubricant replenishment protocol ($p = 0.0006$) on wear rate. Thus, doubling of contact area was associated with a doubling in wear rate despite a twofold decrease in nominal contact stress. Alternatively, the data from these groups can be considered with regard to nominal contact stress. From this vantage point, the increase in contact stress brought about a twofold *decrease* in wear rates. In either case, this change was brought about with no change in normal load.

These findings are supported by a comparison of the pilot group and Group 1A, in which a fourfold increase in area was related to a twofold increase in initial wear rate. This comparison is confounded somewhat by the difference in lubricants in the pilot

group and Group 1A, but it does offer additional experimental evidence for an effect of area on wear rate.

It was possible to gather meaningful friction measurements to compare both Groups 2A and 3A and Groups 2B and 3B. Under protocol A, values were obtained at 0.1 and 0.2 Mcycles, 0.6 and 0.7 Mcycles, and 1.1 and 1.2 Mcycles. At all time points, Group 3A had a higher coefficient of friction than Group 2A (though one nonsensical data point at 0.4 Mcycles was discarded). No effect of time was evident in this experiment, and the difference in friction (Table 6.3.1) was statistically significant ($p = 0.011$). Under protocol B, data were gathered for the first 0.5 Mcycles (i.e., at 0.6, 0.7, 0.8, 0.9, and 1.0 Mcycles. Again, friction was higher for Groups 3B than for Group 3A. There was some change in friction with time (friction increased slightly from 0.1 to 0.3 Mcycles, then decreased over the last 0.2 Mcycles), and the difference between the groups was highly statistically significant ($p < 0.0001$) with time considered as a covariate. Ignoring time as a covariate, the difference given in Table 6.3.1 was still highly statistically significant ($p = 0.0001$). Therefore, in each case, an increase in area brought about an increase in wear rate, and was associated with an increased coefficient of friction. Furthermore, the difference in wear rate was large in each case (approximately a factor of two), whereas the difference in friction was smaller, but still significant.

Effect of Normal Load Independent of Area

Two groups employing the same lubricant replenishment protocol were run using the same nominal contact area (31.7 mm^2) but a twofold difference in load (223 versus 111 N; Groups 2B and 4B in Table 6.3.1). Despite this substantial change in normal load (and nominal contact stress), there was no change in the wear rate ($p = 0.13$, $\beta = 0.30$) or coefficient of friction ($p = 0.17$, $\beta = 0.26$). In Group 4B, friction was measured each 0.1 Mcycle for the first 0.5 Mcycles, then again at 1.4 Mcycle. No change in friction was evident during the course of the experiments. It should be noted that although the difference in coefficient of friction was not statistically significant between these groups, the normal force was different, and therefore the friction force differed significantly ($p < 0.0001$).

It is not appropriate to compare Group 3A with the pilot group because of the different lubricants used, the different duration of the experiments, and the infancy of the methodology used in the pilot study. That the pilot study should be treated separately from later studies is suggested by the very different coefficients of friction obtained for Group 1A and the pilot group when compared to all other groups.

Effect of Contact Area Independent of Contact Stress

The effect of a change in contact area on wear without a concomitant change in contact stress can be examined in two comparisons. First, comparing groups 3B and 4B (Table 6.3.1), we find a doubling of wear rate with a 2.2-fold increase in contact area under similar contact stresses (3.1 and 3.5 MPa). This difference was statistically significant ($p < 0.0001$), as was the difference in coefficient of friction between the two groups ($p = 0.005$). Again, because the normal load was different in these two groups, the difference in frictional *force* between the two groups was also highly statistically significant.

Second, we can compare two groups for which the increase in area was 1.8 fold (increasing from 17.8 to 31.7 mm^2) and for which replenishment protocol A was used (Groups 1A and 2A; Table 6.3.1). Since Group 1 was only tested for 0.5 Mcycles, the comparison between groups must be made at 0.5 Mcycles. In this comparison, in which contact stress was relatively constant (6.3 versus 7.0 MPa), there was no statistically significant difference in wear rate in the first 0.5 Mcycles (p = 0.35, β = 0.13). The coefficient of friction was very different between these two groups ($p < 0.0001$), but the friction force was the same in the two groups (p = 0.62, β = 0.077).

6.4 Discussion

Within the range of loads and stresses evaluated, the wear rate did not increase with normal load as previously reported for metal on plastic articulation[10] and implicit in the use of a "wear factor" in the literature (*e.g.*, Atkinson *et al.*[11]). Because wear increased with contact area and did not change with normal load, wear rate appeared to be inversely related to contact stress over the range of contact stresses tested. However, groups with the same contact area displayed no significant difference in wear rate despite a 2-fold difference in contact stress. Thus, wear rate depended on contact area, rather than contact stress.

These large changes in wear rate were associated with small but significant changes in coefficient of friction. The efforts to measure coefficient of friction met with mixed success. Since the conditions under which friction was measured (and the frequency of measurement) were not easy to control, it is difficult to compare across experiments. Comparisons made between two groups within a single experiment were more reliable. Unfortunately, this limitation makes it difficult to establish whether coefficient of friction or frictional force is a better indicator of wear. The inter-experimental comparisons made between Group 4B and both Group 2B and Group 3B may be more reliable than other comparisons since they were the last experiments performed, and therefore both friction and wear protocols were most well-defined (though friction was still not always measured).

The essential findings of the research were consistent for comparisons among 5 of the 6 experimental groups, with contact areas of 31.7 and 71.2 mm^2. For the sixth group (1A), for which the contact area was reduced to 17.8, there was no concomitant decrease in wear rate in the first 0.5 Mcycles when compared to Group 2A (with a contact area of 31.7 mm^2). This group, performed in conjunction with the pilot study, is difficult to interpret, particularly given the high coefficient of friction measured for this test.

6.4.1 Clinical Wear Factor

The present results are not sufficient to perform regression analysis of wear rate versus nominal contact area, but do show a relationship between increased area and increased wear rate. Because a doubling in nominal contact area resulted in a doubling in wear rate for the results of protocol B, one could consider a linear relationship, such that

$$V = l \times A_a \times k', \qquad \textit{Equation 6.4.1}$$

where V is volumetric wear and l is sliding distance, as defined previously; A_a is nominal or apparent contact area; and k' is a dimensionless variable related to the materials and other test parameters. For this experimental apparatus, each lubricant protocol defined a

single value for k', as shown in Table 6.4.1. Using lubricant protocol B, k' 5.6 x 10^{-9} over the range of area and load tested; using lubricant protocol A, k' was 2.6 x 10^{-9}. It would be worthwhile to conduct additional tests to show whether this relationship holds true over a wider range of applied stresses. Note that, instead of normalizing by sliding distance, one could normalize by number of cycles, determining a parameter with dimensions of mm/Mcycle.

Ideally, one would like to collapse clinical data into a wear factor k' to verify the *in vitro* findings. Although clinical studies suggest a relationship between femoral head size and volumetric wear in clinical retrieval studies,[16,19] it is difficult to simply relate head size to nominal contact area. Therefore, it is not possible to convert these clinical results to a value of k'. Nonetheless, experiments in different laboratories are more appropriately compared using this parameter.

Table 6.4.1 Revised clinical wear factor This table shows the wear rates and wear factors obtained for each experimental group tested for 1.5 Mcycle. All results are given as mean ± standard deviation. Wear factors are calculated as in the text.

Group	*Contact Area (mm^2)*	*Wear Rate (mm^3/Mcycle)*	*Wear Rate $x10^5$ (mm^3/m)*	*k $x10^6$ (mm^3/Nm)*	*k' $x10^9$*
2A	31.7	3.0 ± 1.1	7.4 ± 2.8	0.33 ± 0.13	2.3 ± 0.9
2B	31.7	7.0 ± 0.7	17.4 ± 1.6	0.78 ± 0.07	5.5 ± 0.5
3A	71.3	8.1 ± 2.9	20.3 ± 7.2	0.91 ± 0.32	2.8 ± 1.0
3B	71.3	16.0 ± 2.1	40.0 ± 5.2	1.80 ± 0.23	5.6 ± 0.7
4B	31.7	7.6 ± 0.5	19.0 ± 1.2	1.78 ± 0.11	6.3 ± 0.4

6.4.2 Results in Context of Prior Functional Relationships for Wear of PE

Some of the early laboratory studies showed that PE-on-metal wear rates were very low at low contact stress and exponentially related to contact stress at higher contact stress. Rostoker *et al.* reported that this change took place at a critical stress of 7 MPa, but his tests were unidirectional, and lubricated by distilled water.[13] Rose *et al.*, using serum lubrication, but also using unidirectional motion, reported a critical pressure-velocity product, above which wear increased exponentially;[14] this may have been related to the pressure-velocity limit for polyethylene.[10] Barbour *et al.* found an increase in wear rate with increased area and load.[20] This finding was converted to a "wear factor," which was then independent of contact area, but inversely related to contact stress. It is difficult to correlate these data to the present findings, however, since they were derived from tests using unidirectional motion.

A relationship between contact area and wear rate was suggested by a recent report of Sathasivam *et al.*[2] in bi-directional POF tests. The results showed an increase in wear rate with nominal contact area over the range 50 to 110 mm^2, though still larger nominal contact areas resulted in negligible wear. The authors offered "lubricant starvation" as an explanation for the apparent contradiction between their results and their expectation that wear rate would increase with contact pressure. In their experimental apparatus, the larger pins articulated in such a way that portions of the metal surface were never exposed to lubricant; this phenomenon has been called lubricant starvation.[21] When lubricant starvation occurs, boundary lubricants do not have the opportunity to

adsorb to the metal and protect the PE counterface. The present apparatus does not experience this effect because none of the pins were larger than 10 mm in diameter, yet the trend toward higher wear with higher contact area was still found. It should be noted that although all parts of the metal surface were exposed to serum, the articulating portion of the PE surface was never exposed to serum in this articulating pattern.

The present study offers an alternative explanation for the above findings of Sathasivam *et al.* in a direct dependence on nominal contact area. Estimating k' over the range of nominal contact areas 50 to 110 mm^2, we find $k' \sim 1 \times 10^{-9}$. This result undershoots the present value of 5.5×10^{-9}, but this difference is likely due to differences in experimental apparatus. Sathasivam's apparatus was designed to mimic the articulation of TKA (5° of rotation in conjunction with linear motion), whereas the present apparatus is designed to maximize bi-directional motion. Thus, the present apparatus prevents the alignment of PE, whereas the former work employed a primarily reciprocal motion, allowing PE alignment.[8] It should be noted that wear drops to virtually zero in their articulation below a nominal contact stress of 5.3 MPa (nominal contact area 226 mm^2).

In another study, Saikko and Ahlroos compared 7.1 and 62 mm^2 polyethylene wear faces in a multidirectional device, finding a higher wear rate with increased nominal contact area. They focused their analysis on the higher wear found with greater nominal contact area, though they did note that the higher wear rate was consistent with clinical findings.[5] Calculating a revised clinical wear factor from their study, we find that $k' = 3 \times 10^{-9}$ with the larger pins; k' cannot be calculated for the smaller pins, since their nominal contact area increased with time due to chamfering. The difference between the value obtained from their study and the present study is easily explained by differences in articulating surfaces (they used stainless steel), lubricant protocols and type of motion, but could also be due to a dependence on normal load or other parameters.

Wang also examined the relationship between wear rate and contact stress in a hip simulator.[17] Using different clearances to vary contact stress, he found an inverse power law relationship between maximum contact stress and wear rate. Since load was held constant, this relationship between contact stress and wear rate was similar to the direct relationship between wear rate and contact area in the present study.

These results, in conjunction with the others discussed above, call into question the traditional thinking that increased normal load necessarily leads to higher wear rates in the articulation of metal-on-PE. Furthermore, these results suggest that the use of a "clinical wear factor" to verify laboratory findings may be flawed, since wear rate depends on parameters other than normal load within physiological compressive stresses.

A new model of wear in multidirectional PE-on-metal is warranted – one which accounts for the present results as well as the results of Sathasivam *et al.*,[2] in which virtually no wear occurred at low stresses. Within a physiological range of compressive stress, wear rate increases with increasing contact area. At loads that allow the metal asperities to engage the PE surface, the volume of wear generated appears to increase with contact area. The results of the present study, as well as those from Saikko and Ahlroos[5] and some of those from Sathasivam *et al.*,[2] fall into this range of compressive stress. At still higher applied stress, articulation leads to fatigue and delamination processes. In this range of high contact stresses, increased stress will hasten damage to

the PE components, so a dependence on nominal contact stress is expected. Catastrophic delamination reported in some knee prostheses occur in this range of contact stress.

The nominal contact stresses at which the transitions occur between these three load regimes depend on a number of parameters, including lubricant, loading and motion patterns, and material properties. When running laboratory tests, these parameters should match those found *in vivo* as much as possible.

6.4.2 Wear Surfaces and Lubricant Features

Pin and disk surface morphologies were consistent with those found in clinical retrievals. The significance of the features that appeared as pimples has yet to be determined. That these features were visible on all PE wear surfaces by 0.5 Mcycles warrants their further investigation. While their presence did not affect the mean roughness of the samples and could not be found to affect wear, insight into their formation might shed light on the effects of the wear process on the structure of PE. Based upon the linear relationship between volumetric wear and number of cycles, without any wear-in period, it is unlikely that the changes in PE surface or the presence of PE wear particles after several thousand cycles has any significant effect. This finding is further supported by the linear relationship between volumetric wear and interval between measurements (Fig. 6.3.5). An effect of PE particles would be seen in a change in wear rate (slope) as the length of the interval between measurements increased. It is well-documented that metal particles can cause significant third body wear, but the presence of PE particles appears to have little effect in these tests. Consequently, it is appropriate to ignore the contribution of these particles to the tribology of metal-on-PE TJA.

The gross change in lubricant appearance from transparent and red-brown to a cream color was likely due to changes in serum proteins. The color change likely indicated that proteins were changing conformation (*i.e.*, becoming denatured). The particles found in the lubricant were larger than the volume of PE lost during the interval and therefore too large to be wear particles generated by a transfer film. These, too, were likely protein aggregates. Since these likely protein particles, along with the change in opacity, occurred only with loading and articulation, it appears that they required either frictional heat generation or mixing due to relative motion (or both) for their formation.

As evidenced by the small mass gain of the load soak controls, fluid absorption had little effect on the mass loss of the pins; this finding contrasts with many other reports, in which absorption was of the same order of magnitude as wear. The load soaking most likely made less difference in this apparatus because of the relatively small amount of surface area of each pin exposed to lubricant. This occurred because only a small portion of each pin that was immersed in lubricant, which was made possible by employing individual wells for each pin, rather than a large lubricant bath.

6.4.3 Lubricant Replenishment Protocol

The initial replenishment lubricant protocol had immediately apparent shortcomings. For example, adding water was intended to minimize change of protein concentration due to evaporation, but it was clear that evaporation was not the primary means of changes in serum protein concentration in this experimental apparatus.

Furthermore, adding water did not restore the serum to its original gross appearance, so it was unlikely to return its initial composition. Finally, a solid protein film had adhered to the surface of the well, and, in some cases, to the exposed portion of the disk. Depending on the mode of delivery of water to the well, this protein could be dislodged, and may have served as a solid lubricant, as has been suggested.[22] This mechanism is consistent with the raw data of protocol A: in some cases, pins exhibited wear comparable to pins under protocol B; in other cases, pins lost only half as much mass. Since the wear rate did not change during the course of undisturbed tests, the findings support a change in tribology at a discrete time in the test cycle. For example, a change may have occurred due to solid lubrication by protein aggregates introduced to the articulating surface with the addition of distilled water. That this change occurred only in some couples would explain the lower mean wear rate and larger coefficient of variation using protocol A versus B. No matter the cause, the difference noted in lubricant protocol B represented an improvement over the initial protocol.

6.4.4 Experimental Protocol

The parameters of these experiments were chosen to simulate physiological motion and loading in the hip joint as much as possible. The motion was chosen consistent with bi-directional methodology used by others.[4,7,8] The stresses were chosen to coincide with those found in finite element analysis of the replacement hip.[23] The nominal contact area was limited by the size of the disks and the need to avoid lubricant starvation (from areas of Co-Cr that are never exposed to lubricant). The loads were chosen to bring about appropriate nominal contact stresses given the nominal contact area.

As evidenced by the goodness of fit of a line through the origin, use of a wide range of intervals between measurements did not adversely affect the repeatability of the results. Furthermore, despite the gross changes in lubricant that occurred over the course of 0.3 Mcycles, the wear rate was not affected. This suggests that the mechanisms of lubrication, and consequently, wear, occurring during this test do not change during the interval between measurements.

Previous work suggested that volumetric wear depends linearly on sliding distance (or number of cycles) and normal load.[11,12] Many groups have had to run up to one million cycles before they reach a "steady-state" wear rate (*e.g.*, Saikko and Ahlroos[5]). Using lubricant protocol B, the linear relationship between volume loss and sliding distance extended from the first measurement, rather than after a period of non-linear wear. The magnitude of control absorption was the same as the y-intercept, suggesting that all deviation from volumetric wear directly proportional to sliding distance can be explained by fluid uptake. It appears that effects such as strain hardening or preconditioning PE did not cause changes in volumetric wear rate within the time frame of the present experiments. The goodness of fit of a straight line relating volume loss to sliding distance justifies the use of a "wear rate" defined by the slope of this line. It is essential to turn from volumetric wear to wear rate because it enables comparisons to be made between groups meaningfully in a shorter time frame than a ten million cycle experiment.

Due to a limited amount of lubricant absorption, there was a difference between the 1.5 Mcycle wear rate and the estimate obtained after 0.5 Mcycles. Although this

difference was small under lubricant protocol B, the difference was significant. Under lubricant protocol A, the differences were much larger between the 0.5 Mcycle estimate and the wear rate over 1.5 Mcycles. Due to high variability in the data and relatively small sample sizes, however, these differences were not statistically significant. The present data *do not* justify the use of a 0.5 Mcycle test to determine differences in wear rates, but using lubricant replenishment protocol B, *do* justify the use of 1.5 Mcycle tests to demonstrate such differences. However, this approach should be further explored by additional testing. When comparing tests run for different times, it is necessary to compare the first 0.5 Mcycles of one test to the first 0.5 Mcycles of another.

6.5 Wear Results in the Context of the Conceptual Model

In addition to their primary purpose (to investigate the importance of geometric parameters on wear in PE on Co-Cr articulation), the results of this work can be applied to the conceptual model of wear in TJA as introduced in section 5.9. Specifically, relationships among contact area, normal load, friction, and wear rate are discussed below in the context of this schematic understanding. Based upon this discussion and section 5.9, it is possible to interpret further the present results. In particular, the present results in the context of the greater body of literature support three regimes of loading, with very different relationships between geometry and tribology. These are discussed successively in the articulation of PE and metal through a consideration of POF and sphere-on-flat articulations, as evaluated experimentally.

6.5.1 Wear and Real Contact Area

In traditional approaches to wear, abrasive and adhesive modes of wear are treated as proportional to the real area of contact. In traditional engineering tribology, the abrasive and adhesive wear equations (2.4.2 and 2.4.3) both begin with

$$V = l \times A_r \times C, \qquad \textit{Equation 6.5.1}$$

where V is volumetric wear, C is a constant related to the particular surfaces and wear mechanisms, and l is sliding distance.[21,24] These relationships are derived straightforwardly from a consideration of the interaction between surfaces. Adhesive wear depends on chemical interaction between two surfaces, and so specifically requires direct contact between surfaces. Abrasive wear, likewise, occurs when an asperity from the metal surface engages the PE surface. This, too, depends on the real area of contact.

Traditionally, tribologists have moved from Equation 6.5.1 to an equation relating wear to normal load. By assuming that load is borne by plastic deformation (as in metal-on-metal asperity contact (Equation 5.9.3), Equation 2.4.1 can be generated, relating volumetric wear linearly to normal load This equation has traditionally been used in TJA despite the fact that substantial load is borne by elastic deformation (rather than plastic) in metal-on-PE articulation. The present results suggest that this equation is not appropriate for analysis of metal-on-PE arthroplasty.

A more appropriate analysis employs Equation 6.5.1, taking consideration of the true determinants of A_r. As is discussed below, in the low and intermediate loading regimes, in which abrasive and adhesive wear dominate, wear is principally determined by A_r. Recall the equations governing elastic and plastic deformation in PE-on-metal

articulation from section 5.9.3. For Hertzian contacts (including basically anything but flat-on-flat),

$$A = A_r + A_l \sim W^n, \text{ where } 0.67 \leq n \leq 1; \qquad \textit{Equation 6.5.2}$$

for flat-on-flat contact, this relationship is not valid, and the governing inequality is $A_a \geq A_r + A_l$. Since the presence of a boundary lubricant interferes with both modes of wear, only A_r contributes to wear. Again, though it is an oversimplification to state that no wear occurs when lubricant is present, it is useful for the purposes of illustration to consider A_l and A_r in this fashion. It has already been discussed how a good boundary lubricant reduces A_r; in section 5.9.2, it was discussed how a good lubricant is manifest in reduced friction. Now it can be seen how a good lubricant, by reducing A_r, also can reduce wear in PE on Co-Cr articulation. Additionally, a stronger relationship between friction and wear is now seen, since both are directly affected by A_r. Now, in addition to intuitive arguments, historical utility, and empirical evidence, there is a mathematical framework (albeit a semi-quantitative one) to relate the two tribological measures.

In the next three sections, I discuss the effect of normal load and contact area in the context of three different load regimes. These three regimes are related to both the present results and the greater body of work in the literature. As described above, adhesive and abrasive modes of wear are principally determined by the real area of contact, which, in turn, is affected by other parameters, depending on the stress regime.

6.5.2 Effect of Load and Area under Low Stress

As shown below in Fig 6.5.1, load may be sufficiently small that boundary lubricant almost completely covers the metal surface, preventing surface to surface contact and minimizing deformation of the PE surface. Given an appropriate geometry, fluid film lubrication may even occur, preventing wear altogether. In the POF case, under low sliding speeds, it is more likely that a small number of asperities on the metal surface engage the PE surface, and little wear occurs. Under these conditions, A_r is very low, and therefore, wear rate is very low.

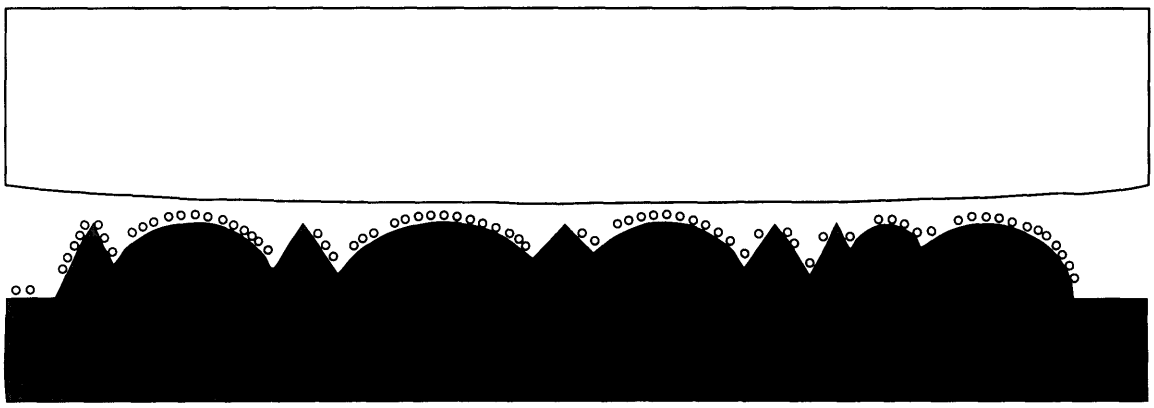

Fig. 6.5.1 Schematic of PE-on-metal articulation under low load Under low load, there is little deformation of the PE surface, and little interaction between the surfaces. Little wear occurs under these circumstances. As per the discussion of section 5.9, the boundary lubricant shown here is small and globular, rather than linear.

Although the present experiments do not enter this regime of articulation, laboratory studies and clinical findings in non load-bearing joints support its existence.

In total shoulder and elbow arthroplasty, PE wear leading to osteolysis is greatly reduced. These joints bear much lower loads than the hip and knee do, suggesting that reduced load can be related to very low wear. Furthermore, under Sathasivam's TKA POF protocol, nominal contact stresses below 5.3 MPa resulted in no measurable wear even though the lubrication was most likely mixed or boundary.[2] In contrast, the present results showed measurable wear even at 3.1 MPa nominal contact stress under THA-like bidirectional articulation. Thus, the evidence supports a transition form very low wear to measurable wear dependent not only upon the nominal contact stress, but also upon the pattern of articulation (with perpendicular bidirectional motion leading to the greatest amount of wear). This finding is consistent with that reported by Wang in his "unified theory of PE wear."[8] Although the nominal contact stresses listed here do not indicate the actual applied stresses, they give a general indication of what stress states would be required to eliminate wear in THA and TKA.

Within this regime, A_r may be determined on the level of individual asperities bearing most of the load. At these asperities, A_r may be governed by Equations 5.9.3 and 5.9.5 (elastic and plastic deformation). Thus, increasing load within this regime leads to increased wear, since higher loads bring about a greater area of contact. Likewise, increasing area may lead to increased wear, since area of contact is increased. In this regime, however, wear rate is low relative to wear rate higher stress regimes. In sphere-on-flat experiments (*i.e.*, friction tests), low loads lead to a similar situation. A_r again is low, so F_f is very low. This geometry prevents fluid film lubrication, however, so F_f decreases with W according to the power law relationship governed by Equations 5.9.3 and 5.9.5. Since W is low, μ may be relatively high even if F_f is low. Friction in this regime still likely depends on geometry and surface interactions, though both good and bad boundary lubricants may be effective, since the load may be low enough for even a poor lubricant to bear. Equivalent lubrication by good and bad lubricants is supported by the friction results at low load presented in Chapter 5.

6.5.3 Effect of Load and Area under Intermediate Stress

The present experiments relate more directly to higher loads, in which much of the PE surface engages metal asperities, as shown in Fig. 6.5.2. In this intermediate regime, articulation is more consistent with the illustrations of section 5.9. That is, the surfaces approach each other more closely, PE surface deformation increases, and both A_r and A_l increase. Under these conditions, abrasive wear and adhesive wear may occur at much higher rates than in the first regime. The conformity of the surfaces in POF articulation is sufficient that $A_r + A_l$ approaches A_a. Therefore, increased load brings about little increase in $A_r + A_l$, though asperity engagement may deepen and applied stresses may increase on the PE surface. These factors have little effect on A_r, however, which is the principal determinant of wear. Therefore, within this intermediate regime, wear does not increase substantially with load. The wear findings of this chapter are consistent with this relationship between load and PE wear rate.

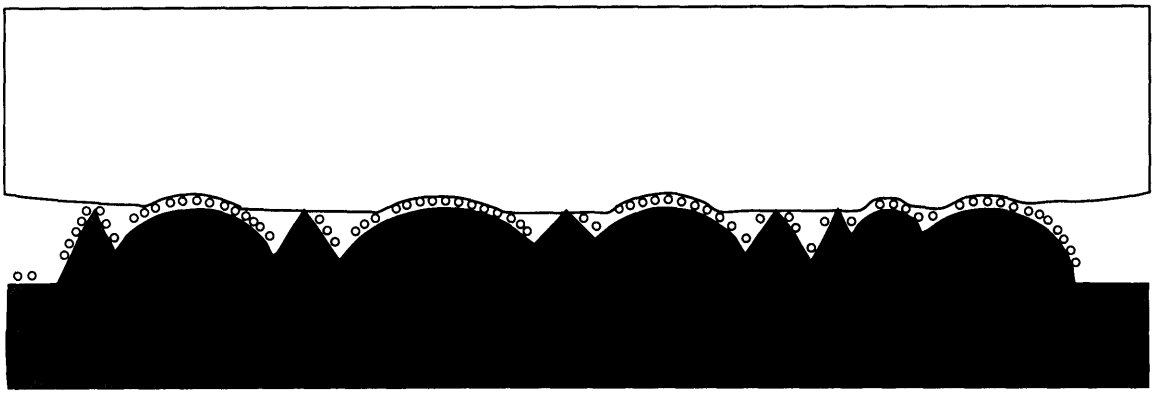

Fig. 6.5.2 Schematic of PE-on-metal articulation under intermediate load Under higher load, real contact increases dramatically between the surfaces. The role of a good boundary lubricant becomes increasingly important, because it determines the relative magnitude of A_l versus A_r. The boundary lubricant shown here is doing well to reduce real contact between the surfaces.

On the other hand, changing the apparent area of contact has a significant effect on tribology. Since the surfaces are so conforming,

$$A_r + A_l \approx A_a, \qquad \textit{Equation 6.5.3}$$

and increasing A_a has a substantial impact on wear. In fact, for a given lubricant, wear in this regime is principally determined by A_a. As demonstrated in the results of this chapter, an increase in A_a brings about an increase in wear rate even though it results in a decrease in nominal contact stress. This comes about because more asperities are engaged in this intermediate loading state as area increases (Fig. 6.5.3). On the other hand, if the increase in apparent contact area reduces applied stress sufficiently, it could shift the articulation back into the low stress regime, reducing wear dramatically.

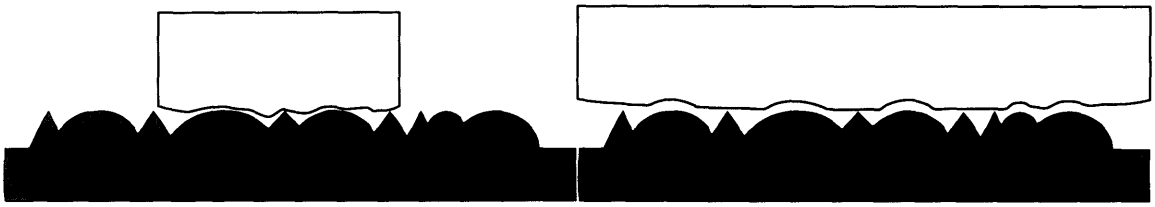

Fig. 6.5.3 Comparison of small (left) and large (right) PE pins articulating on metal Although the smaller pin results in higher stress, and therefore greater deformation of the PE surface, the larger pin results in the engagement of more asperities, and therefore more wear. In this illustration, the boundary lubricant is not shown for simplicity.

Since both friction and wear depend on A_r, the schematic predicts that area and load would have the same effect on friction and wear. Under the same load, as area increased, friction increased (Group 2A versus 3A and 2B versus 3B). Importantly, these comparisons could be made for groups run simultaneously, and were therefore less subject to variability that may have occurred in the conditions surrounding friction measurement in a given experiment. An additional comparison showing the same general trend is that between the pilot group and Group 1. Each of these experiments showed a consistent increase in both friction and wear concomitant with an increase in nominal contact area.

It is worth noting that although a doubling in A_a led to a doubling in wear rate (consistent with Equations 6.5.1 and 6.5.2), friction increased by a much smaller margin (inconsistent with Equations 5.9.2 and 6.5.2). By using a device that can measure friction and wear concomitantly (more reliably than the OrthoPOD), it may be possible to determine empirically the relationships among these parameters with greater accuracy. Regardless of more exact quantitative relationships, the finding that a small increase in friction correlates with a large increase in wear is consistent with the results of Chapter 5 comparing water and bovine serum. This finding further supports the claim that small differences in friction shown in Chapter 5 herald clinically significant differences in wear.

The schematic illustration also predicts no increase in F_f with normal load. Although coefficient of friction was the same under different loads, F_f was higher under increased load. However, since only one load could be applied per six-station experiment, it was not possible to compare concurrent friction measurements under multiple loads. As documented above, the friction results were difficult to compare across experiments because of confounding variables. It would be worthwhile to re-examine the relationships among friction, wear, and normal load using a more sophisticated instrument.

Within this intermediate loading regime, the lubricant can greatly impact both friction and wear. Friction in this regime occurs as described in some detail in section 5.9, wherein a better boundary lubricant provides reduced coefficient of friction and reduced wear through a shift in the balance of A_l/A_r. Decreasing A_r leads to a decrease in both friction and wear. The importance of boundary lubrication increases when high conformity enables a good boundary lubricant to cover much of the articulating surface. In the sphere-on-flat case, the contact area is flattened by high stress, thus allowing a large potential area to show good or poor boundary lubrication. Thus, the sphere-on-flat geometry was ideal for measuring the effects of boundary lubricants on the tribology of TJA. The effect of lubricant in this regime is consistent with the finding of a different value of k' under two different lubricant protocols. In particular, we can relate k' in Equation 6.5.1 to C in Equation 6.4.1 through the ratio A_r/A_a, which for a given geometry, load, and pair of surfaces, depends on the lubricant. Thus, a lubricant that decreases the real area of contact relative to the apparent area of contact also reduces wear in this regime.

Interestingly, the schematic as described herein is consistent with other empirical features of PE wear. For example, it is clear from the illustrations that rougher surfaces (*i.e.*, sharp asperities) decrease the opportunity for boundary lubrication to offer protection from wear. This prediction is consistent with the empirical relationship between roughness and PE wear.[25,26] Additionally, suppose we replace PE with a material that requires less shear stress to generate a wear particle (*i.e.*, reduced τ_w). Obviously, wear rate increases, assuming all other parameters are maintained. In this schematic illustration, it is also evident that the decrease in τ_w reduces F_f (Equation 5.9.2). Thus, the schematic illustration explains the increase in wear with PTFE despite reduced friction under many conditions.

6.5.4 Polyethylene Damage under High Stress

Throughout the intermediate region, local stresses exceed the yield strength of PE, enabling plastic deformation of PE. As load continues to increase, however, more catastrophic failure of PE may occur. As defined by Suh, delamination wear begins with crack nucleation, requiring stresses exceeding the yield stress of PE over a region of material.[27,28] Thus, the critical dimension is the size of the plastic deformation zone, rather than a maximum stress of some kind. Although the damage processes that occur in PE in this articulation are somewhat different than Suh's description of delamination, a similar threshold likely exists to initiate such damage. There is no evidence of such damage occurring in either the friction experiments of Chapter 5 or the wear experiments of the present chapter, but has been shown to occur in some TKA designs.[29] Subsurface crack nucleation under high load is illustrated schematically below in Fig. 6.5.4.

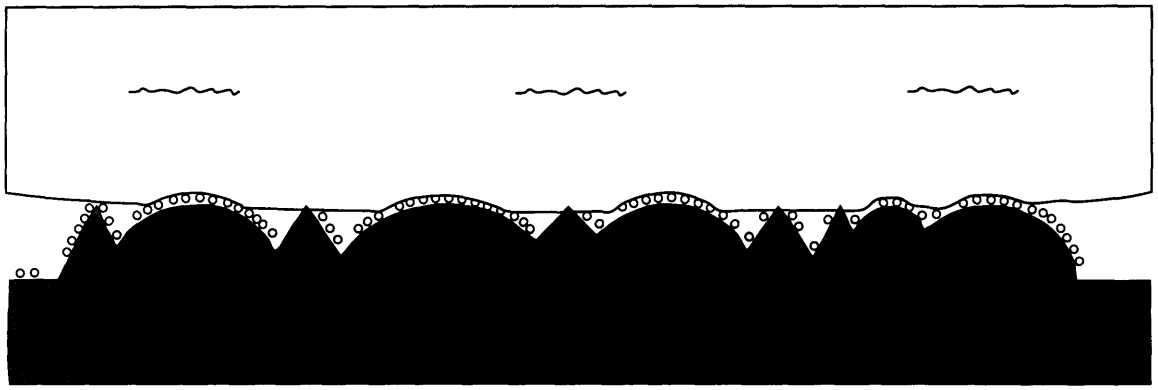

Fig. 6.5.4 Schematic of PE-on-metal articulation under intermediate load As load increases still further, stresses may exceed a yield criterion over a region larger than a critical size, leading to permanent damage to the PE. This damage occurs largely independently of the effects of a boundary lubricant.

Different parameters are likely determinant of wear in this regime than in others. For example, boundary lubricants can do little to protect against such damage. The rate of wear is likely determined by the geometry of the articulation and loading conditions, but the relationships would not be expected to be linear. Wear in this regime would not depend as much on bidirectional articulation as wear under low stresses would, since the damage happens on a scale larger than the individual PE chains that may become aligned in unidirectional motion. A transition from a low wear regime into this regime was most likely the source of the exponential relationship between nominal contact stress and wear rate in unidirectional POF tests[13,14] discussed previously.

6.6 Conclusions and Relevance

In summary, this study showed an increase in PE wear rate associated with an increase in nominal contact area for the articulation of PE on Co-Cr. Wear rate was not affected by normal load. These findings sharply contrast with the conventional use of a clinical wear factor to relate normal load to volumetric wear rate. In conjunction with our schematic model for PE-on-metal tribology, these findings have implications for both total hip and knee arthroplasty. In knee arthroplasty, it suggests that more conforming prostheses, with higher nominal contact areas, will generate more volumetric wear than their less conforming counterparts, unless they can reduce contact stress sufficiently to reach the first regime of contact stress (*i.e.*, low stress regime). In hip arthroplasty, it

suggests that larger femoral heads are detrimental from the standpoint of generation of volumetric wear. This may be particularly relevant now that cross-linked PE has revived the move toward larger femoral heads to increase stability. The effect of increased nominal contact area must be considered carefully before such recommendations are made, considering that cross-linked PE also undergoes abrasive wear, albeit with lower wear rates.

Furthermore, the friction measurements, though incomplete, generally supported a connection between coefficient of friction and wear rate as described by the schematic illustration of sections 5.9 and 6.5. More importantly, however, they supported the use of the friction assay of Chapter 5 to determine differences in tribology, in that large differences in wear in extended tests were indicated by small differences in friction in rapid tests. These findings further support the main thrust of this thesis, that variability in joint fluid affects the tribology of TJA.

6.7 References

A portion of this work has been published previously,[30] though all the friction data and much of the discussion included in this chapter are new.

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CHAPTER 7

CONCLUSIONS

7.1 Introduction to the Conclusion

As the opening sentence of this thesis states, my primary intent in this research was to analyze joint fluid in the context of its effect on the tribology of TJA. In the first two studies, the properties and composition of joint fluid were measured. A third study evaluated the effect of joint fluid on friction in TJA. The final study evaluated wear in TJA as it relates to certain geometric parameters, but did not specifically evaluate joint fluid or its components. The purpose of this closing chapter is to summarize and synthesize the significant results of these studies in the context of the greater body of knowledge on tribology in total joint arthroplasty.

In this concluding chapter, the application of POF articulation to TJA is discussed. Then, the essential results of the four major experimental thrusts of this thesis are discussed in the context of each other and relevant work by others. Much of this discussion has taken place in previous chapters, particularly in sections 5.9 and 6.5, and is not repeated here. Practical application of this work to the treatment of joint disease is discussed. Finally, based upon the results of this thesis, future experimental work in this field is recommended.

7.2 Approaching Clinical Articulations

The experimental and illustrative body of this thesis discusses primarily simple lubricated sliding between metal and PE, but clinical reality is more complex. For example, the experiments and illustrations of this thesis all involved sliding contact. Non-sliding articulations, such as the rolling that occurs in TKA, may not require an analysis completely separate from the one given for sliding. Many of the same principles apply, though perhaps different parameters may dominate (such as extensional viscosity rather than shear viscosity, for instance). The high impact actions of jumping, bracing oneself in a fall, or walking up steps, likely require a separate analysis from the one above. These actions may result in very high local stresses, and so may not be amenable to boundary lubrication. They may be initiating actions in a delamination-type wear mode, as described in section 6.5.4. PE may be protected by joint fluid flow properties in these cases, however. In high speed motions, joint fluid may absorb the load or at least distribute it over a larger area of PE, thus reducing the stresses applied to the PE. Given the variability in joint fluid flow properties, one could argue that some patients are at greater risk of damage due to such a blow, but this thesis does not provide evidence to do more than suggest this possibility. I have not seen a study that scientifically examined an association between traumatic events in TJA and wear-related failure.

Furthermore, the articulation may lack lubricant altogether. The absence of lubricant would affect all aspects of tribology, underscoring the importance of the quantity of joint fluid. Simply dissipating heat, removing particles, and preventing particle agglomeration are important functions of lubricants (c.f. section 2.4.6) depending on quantity, not quality. None of these aspects of lubrication are discussed in the illustrations, since it is assumed that lubricant is present in this joint. If there is so little fluid as to not perform these functions, wear is undoubtedly accelerated, and clinical outcome much worse than if lubricant is present.

7.2.1 Stress Regimes in Clinical Articulations

Even when examining relatively simple walking patterns in clinical articulation or in simulator studies, the sliding motion is more complex than the bidirectional POF motion, and much more complex than the unidirectional friction tests. By consequence, the joint does not simply "exist" in one of three stress regimes, as most POF articulations do. Many loading conditions exist simultaneously in different parts of the replacement joint, and these may change throughout the gait cycle. For example, the low stress regime (section 6.5.2) always exists in parts of the joint. In TKA, since there is less conformity than in THA, there is a large portion of the joint over which little load is borne and little wear can occur. In THA, though there is a greater area over which contact occurs, much of this contact is under low stress for at least part of the gait cycle. Furthermore, the shape of the bidirectional motion differs on different points on the femoral head (or acetabular cup).[1] The stress required to depart from this regime depends on the shape of bidirectional motion, as suggested by Wang[2] and as suggested by the differences between the results of Chapter 6 and the report of Sathasivam *et al.*[3]. Thus, the relationship between stress distribution and volumetric wear is complex. Nonetheless, using the schematic illustration given in these chapters, one could use finite element analysis to determine the likely wear pattern on an acetabular cup or tibial plateau given this three regime description of PE wear.

Although further study is appropriate, some comments can be made already regarding TJA articulation. For example, the high stress regime is the one of greatest concern, and must be avoided at all costs in TJA. Within this high regime, wear processes are initiated that may continue to occur even if stresses decrease at a later time. These processes lead to rapid and catastrophic failure. Although the necessary conditions to avoid this type of wear have not been completely elucidated, PE thickness[4] and surgical alignment[5] are two factors that can lead to wear in this regime. High stresses have been associated with catastrophic damage in TKA, often occurring in particular designs.[6,7]

It has been reasoned by some designers that if some increased conformity leads to reduced damage, a greater increase in conformity will reduce wear still further. Efforts have been made in TKA to reduce wear using more conforming geometries, but these have met with mixed success. For example, mobile bearing knees introduce an intermediate surface similar to a meniscus to provide increased conformity and distribute load more evenly. Although this design change has decreased the catastrophic failure due to rapid wear (characteristic of the high stress regime),[6] osteolysis has become more prevalent. For example, one study of failed knee prostheses found that 47% of failed mobile bearing knees showed radiographic evidence of osteolysis versus only 13% of failed fixed bearing knees.[8] Likewise, wear debris in mobile bearing knees is smaller and more granular, consistent with adhesive and abrasive debris from THA, whereas fixed bearing knees generated larger particles consistent with delamination-type wear.[9] Given the illustration described in this thesis, a reduction in contact stress generated by increasing the area of contact does not decrease PE wear *a priori*. Unless such a design decreases stress sufficiently to achieve the low stress regime, one would expect this increase in area to increase adhesive and abrasive wear. This prediction is consistent with the clinical observations described above.

From a TJA design standpoint, one could potentially achieve the low stress regime of tribology by modifying the geometry of TJA. For example, once a quantitative relationship among shape of articulation, stress, and transition from low to intermediate loading is developed, prostheses could be designed to remain in the first regime throughout the gait cycle. Such a regime might be most simply achieved by fluid film lubrication in THA. In this case, the flow properties of the lubricant are the most relevant lubricant parameters. In TKA, since the constraints of the joint greatly reduce the opportunity for fluid film lubrication, radical design changes may be required to achieve a low stress articulation.

7.2.2 The Role of Joint Fluid

At the outset of this thesis, it was proposed that composition of joint fluid and flow properties of joint fluid would play independent and important roles in the tribology of TJA. This finding still appears to be the case. Thus, two roles of joint fluid are accepted. As shock absorber and fluid film lubricant, the flow properties of joint fluid are important to protecting the replacement joint. The strong correlation among parameters in joint fluid supports the use of a single parameter, such as η, to describe its flow properties. The second role, as boundary lubricant, depends on the composition of joint fluid, and not on its properties. Since individual components vary in a manner not clearly related to each other, these cannot be estimated by a single parameter, though the friction assay suggests that coefficient of friction could be a single meaningful parameter. Finally, quantity of fluid also must be considered. Neither mode of lubrication can occur when fluid is not present in the joint space, so quantity must be sufficient to enable lubrication.

Joint fluid has the greatest effect in the low and intermediate stress regimes. Joint fluid affects the real area of contact in these articulations, thus leading to an effect on adhesive and abrasive wear. The importance of lubricant is underscored in numerous laboratory wear tests, (*e.g.*, Lewis *et al.*[10]). In the present thesis, a friction assay showed that one or more components of joint fluid reduce A_r under high loads relative to water, leading to lower friction and indicating reduced wear. At lower loads, the components of joint fluid responsible for boundary lubrication may determine whether an articulation exists in the low stress or intermediate stress regime. The low stress regime implies that the boundary lubricant is providing maximum protection to the surface, and little wear is occurring. It should be noted that, unlike in POF tests, a particular point on a clinical articulation may shift from low to intermediate stress and back during the gait cycle. By consequence, a better lubricant may increase the fraction of the cycle during which wear does not occur for a given point on the PE surface. Thus, a better lubricant may greatly affect wear in PE on Co-Cr TJA. Finally, as stated above, a boundary lubricant has little effect on PE damage in the high stress regime, but a viscous and elastic fluid could offer some protection under high-impact loading.

7.2.3 Summary

The following series of questions can be used to conceptually evaluate wear in TJA. The first question to ask is whether fluid film lubrication occurs. Answering this question requires knowledge of the flow properties of joint fluid and the geometry of the articulation (including roughness, load distribution, and articulation pattern and speed).

If fluid film lubrication occurs, there is a separation between surfaces and therefore no problem of PE wear. If fluid film lubrication does not occur, the next question is whether articulation occurs in the high stress regime. This is defined by the geometry of articulation (including material properties, thickness, and load distribution). In the high stress regime, catastrophic PE damage occurs, leading to rapid failure. Assuming articulation manages to avoid these two extremes, adhesive and abrasive wear are likely the dominant modes of wear. Wear is governed by the model described in sections 5.9 and 6.5, with wear proportional to A_r for sufficiently high stresses. Using this model, the geometry of articulation is important, since roughness, A_a, bidirectional motion, and sliding distance all have important effects. Additionally, the quantity and composition of the lubricant are essential, because they determine the relative balance of A_r and A_l.

7.3 Definitive Experimental Results Presented in this Thesis

Some work in this thesis stands alone, while other work requires additional analysis to be properly applied. For example, the results of flow properties study require no further interpretation. Now that they have been determined, one could begin to assess the affect they have on tribology of joint fluid. Many researchers have discussed the relative importance of fluid film lubrication in THA, particularly through EHD lubrication, even before I reported the flow properties (cf. section 2.2.1). For these studies, joint fluid bulk viscosity in TJA was estimated from the flow properties reported in healthy and diseased joints. Furthermore, if additional geometries and combinations of materials are considered, it is well understood how these will affect fluid film lubrication. In particular, more compliant materials will enhance EHD lubrication, and smoother surfaces will reduce the gap required to maintain a fluid film. Certainly, additional meaningful work could be conducted in the area (*e.g.*, extensional viscosity, small gap rheology), but the work in this thesis requires no additional interpretation to be utilized.

Unlike the rheological work, the work in Chapter 4 describing the composition of joint fluid in TJA, as summarized in Tables 4.5.1 and 4.5.2, cannot be applied usefully without the additional experimentation of Chapter 5. The primary relevance of joint fluid composition in TJA relates to its specific interaction with replacement joint materials. Thus, the examination of its effect on boundary lubrication, as given in Chapter 5, greatly increases the relevance of Chapter 4. These chapters together show the variability in HA, protein, and phospholipid in joint fluid and how the variability affects the tribology of PE-on-metal. Evidently these components are overshadowed by another component in the tribology of PE on Co-Cr articulation, but this could not be known until the work was completed.

Likewise, Chapter 5 demonstrated that joint fluid generates variable friction in PE on Co-Cr. Although historical, empirical, and intuitive arguments are put forth for a relationship between friction and wear, the results and subsequent discussion of Chapter 6 are necessary to provide experimental and theoretical support for the association between friction and wear in this articulation. In particular, the results of Chapter 6 associate small differences in friction with large differences in wear. Thus, the two chapters work together to build toward a more significant result.

7.3.1 Summary of Findings

The essential results of my research can be summarized as follows.

- The viscous and viscoelastic properties of joint fluid vary widely among patients undergoing TJA. Summaries of the ranges of these properties in patients undergoing TKA and revision TKA are given in Tables 3.5.4 and 3.5.7.
- Protein, phospholipid, and HA concentration of joint fluid each vary widely among patients undergoing TJA. A summary of the ranges of these components is given in Table 4.5.1.
- Friction between Co-Cr and PE lubricated by joint fluid sample varies widely from sample to sample, but friction cannot be predicted by total protein, phospholipid, or HA content of the lubricant.
- Within a physiological range of stresses, wear of PE on Co-Cr depends on nominal contact area, and is independent of normal load.

7.3.2 Findings Regarding Joint Fluid

The four principal findings lead to the primary conclusion of this thesis, which can be summarized in three words: "joint fluid matters." This thesis provides substantial support for the claim that joint fluid is a principal determinant of tribology in TJA within a physiologically relevant stress regime. Future studies may investigate in more detail the extent to which variation in joint fluid explains variability in clinical outcome. Additionally, further study may identify which specific components of joint fluid are determinant of TJA tribology. A few additional findings of this thesis relate specifically to friction and wear of PE and contribute toward further identification of the determinants of TJA tribology in joint fluid. These are:

- HA and albumin & γ-globulin increase the friction between PE and Co-Cr relative to saline. Phospholipid does not have an effect.
- Distilled water and saline provide fairly good boundary lubrication of PE on Co-Cr, but bovine serum and certain joint fluid samples performed better. Other joint fluid samples performed worse than water in a manner consistent with their protein and HA content.
- The important component in bovine serum no longer functions after protease digestion.

These results point to one or more components of joint fluid performing a boundary lubricant function in this articulation. The most likely candidate for this lubricant is a protein that has not yet been identified.

7.3.3 Findings Regarding Determinants of Wear in TJA

The results of the wear and friction studies have been considered in light of a traditional model for metal-on-metal boundary lubrication to generate an illustrative model of tribology in PE-on-metal. This model has been used to explain the results, and can be used to guide further study. An ideal model would describe the wear of PE in TJA quantitatively in terms of all relevant parameters. At this time, a quantitative model of this kind cannot be constructed, either from the literature or from present experiments.

7.3.4 Additional Findings

In addition to the results regarding boundary lubrication by joint fluid, the thesis has demonstrated a number of essentially independent observations that do not build toward a greater result. For completeness, a list of the meaningful and independent results is given here:

- The viscous and viscoelastic properties of joint fluid differ among patients undergoing primary arthroplasty compared to those undergoing revision arthroplasty.
- The flow properties of joint fluid from patients undergoing TJA and revision arthroplasty are similar to properties previously measured in synovial fluid from patients with OA.
- The flow properties of joint fluid are quite different from those of bovine serum, which is typically used to simulate joint fluid in laboratory wear tests.
- Protein concentration is higher and HA concentration lower among patients undergoing revision TKA as compared to those undergoing primary TKA.
- The HA, protein, and phospholipid content of joint fluid from patients undergoing TJA and revision arthroplasty are similar to those previously measured in synovial fluid from patients with OA.
- Variation in HA concentration (and to a limited extent, molecular weight) correlates well to the variation in joint fluid flow properties, especially viscoelastic ones. Interactions with other molecules (likely proteins) increases steady shear viscosity, particularly at low shear rates.
- HA concentration correlates inversely to protein and phospholipid concentration in TKA (*i.e.*, as HA content increases, content of the other components decreases).
- The optimal conditions for distinguishing among lubricants for PE on Co-Cr using a friction assay include high loads and small contact area, using a spherical-tipped pin.
- PE on Ox-Zr has a lower coefficient of friction than PE on Co-Cr when lubricated by bovine serum or by individual components of joint fluid.
- Friction between PE and Co-Cr decreases over the first three hours when lubricated by bovine serum.
- Small but significant changes in PE on Co-Cr coefficient of friction are associated with large differences in PE wear rate.

7.4 Benefits of the Research

Upon completion of this thesis, I now evaluate the practical utility of the research that has been conducted in terms of the anticipated benefits laid out in section 1.1.2.

7.4.1 Joint Fluid Lubricity Assay

One long-term goal of this thesis was to develop an assay to determine the quality of joint fluid before or during surgery. This technology would enable more precise prognoses, and could direct medical care. For example, a patient whose fluid is poorly suited to TJA might choose to defer joint replacement to reduce the risk of prosthesis failure. Alternatively, it may be shown that particular combinations of prosthesis materials are well-suited to a certain fluid constitution. Thus, an assay may show that a

particular type of prosthesis leads to a better outcome for a particular joint fluid composition.

A friction assay, such as the one used in Chapter 5, could perform this function. Chapter 5 of this thesis demonstrated that variation in joint fluid tribology can be found using a simple friction apparatus. Synovial fluid could be obtained at TKA as described in section 3.2, or aspirated from the joint prior to arthroplasty. Aspiration of the joint is performed regularly to diagnose disease, so to do so for the purpose of assaying the lubricity of the fluid would not alter the patient-doctor interaction.

The utility of this test presupposes that short term friction measurements correlate with long-term wear results. Although both theoretical and empirical arguments for a relationship have been put forth in this thesis, comprehensive tests are needed before such an assay could be used clinically. Furthermore, the use of a friction assay as described above requires that the lubricating ability of synovial fluid before surgery correlates with that of joint fluid throughout the life of the arthroplasty. This has not yet been demonstrated. If the lubricating ability of joint fluid does vary with time (as might be expected, given the variability shown in this thesis), the clinical application of the assay would be different. Instead of the use described above, a clinician may use the assay when aspirating an effused joint, as an estimate of the *current* lubricity of the patient's joint fluid. Thus, the joint fluid assay may have prognostic value as a periodical measure of lubricating ability, with the understanding that joint fluid varies with time.

The current results do not support the use of a biochemical assay for lubricating ability. Specifically, total protein, HA, and phospholipid content has been shown to be poor indicators of joint fluid lubricating ability. Once one or more lubricating components are identified in joint fluid, this possibility can be reconsidered. If all relevant lubricating mechanisms and molecules were fully understood, a biochemical assay would be superior to the mechanical one because the mechanical (friction) assay is more susceptible to variations in surface properties and the effects of contaminants.

7.4.2 Joint Fluid Simulation

Determining the principal components of joint fluid was an important first step in constructing a synthetic joint fluid for use in TJA wear test. Unfortunately, since the major components of joint fluid have been excluded as principal boundary lubricants, it may be a component present in small quantities that provides essential lubrication. Additionally, as this thesis and other work have shown, the effect of individual components of joint fluid on the tribology of TJA varies based upon the materials. Therefore, even if the critical boundary lubricant for PE on Co-Cr is found, this component may not adequately reproduce physiological tribology for some other combination of materials. Likewise, even though we have shown, for example, that phospholipid has no effect on friction of PE on Co-Cr, it would be appropriate to include phospholipid because if might affect the tribology of some other materials combination. Thus, a fluid that adequately simulates joint fluid for tribological testing on all combinations of materials would be extremely complex, with perhaps dozens of components.

Furthermore, the variability of joint fluid casts doubt on any single fluid choice to replicate the tribological environment of TJA. Thus, even when the boundary lubricant is

identified, the evidence of Chapter 5 shows that it is present (or functional) in some but not all joint fluid samples. Thus, it would be most appropriate to employ a variety of lubricants, each containing variable amounts of the relevant tribological components of joint fluid for a particular couple. I suspect, however, that few researchers are sufficiently interested in the effect of joint fluid to expand their wear simulations to include a complete battery of joint fluid compositions. Thus, an improved understanding of the relevant components for a given couple would be very helpful. For example, though phospholipids have no impact on PE on Co-Cr, they likely lubricate metal-metal articulations *in vivo*. Thus, a wear test evaluating a new metal-on-metal prosthesis should control and record the phospholipid concentration of the lubricant to enable comparison with other experiments and to determine which patients might benefit most from such prostheses. Careful control and reporting of lubricants used in tribological studies will ease the interpretation of present experiments in the future, as our understanding of TJA tribology improves.

7.4.3 Injectable Agents for TJA

Once the agent(s) responsible for lubrication of TJA are found as described above, it will be necessary to examine how to maintain their presence in joint fluid. As in viscosupplementation, agents can be introduced to the joint to stimulate the endogenous production of lubricating molecules. These agents may even be part of the implant itself. Application of such technology hinges on the identification of the lubricating agents.

7.4.4 Intelligent Tribological Design

The present research does take important steps towards understanding the interplay of joint fluid composition and lubrication. It is clear that, even for a single type of PE, the choice of metal counterface affects the relationship between joint fluid components and friction. Several components of joint fluid have been ruled out as important contributors to the lubrication of PE. Furthermore, the importance of boundary lubrication by water, and the potential to interfere with such lubrication, have both been suggested by this research. There is still much unknown about this lubrication, however. Boundary lubrication by water has not been fully elucidated, and the essential component(s) responsible for the boundary lubrication sometimes superior to water have not been found. The mechanism of this lubrication has not been found. Answering these questions will aid intelligent tribological design of materials for TJA.

From the current studies, several recommendations can be made, however. First, if it can be done without reducing the wear resistance of PE, a more compliant surface would increase the opportunity for a boundary lubricant to act as a protective layer between surfaces. A metal counterface that resists scratching (roughening) also increases the opportunity for a lubricant to protect against surface-surface contact. Finally, increasing contact area to decrease stress may not be the answer to reducing the generation of PE wear particles. Increasing contact area only has a positive effect if it reduces stress sufficiently to change regimes (from high to intermediate stress or from intermediate to low stress).

7.5 Recommendations for Future Research

In light of these continued research goals, there are four areas in which continued study could be directly helpful in the field of TJA tribology.

7.5.1 Identify the Boundary Lubricant

The boundary lubricant for PE on Co-Cr in joint fluid should be identified. This has obvious importance in PE on Co-Cr prostheses, but may also be relevant in other hard-on-soft prostheses. There are several approaches that may be of value in this effort. Individual proteins in joint fluid can be identified using electrophoresis. Then perhaps a more meaningful correlation can be drawn between joint fluid composition and friction. Additionally, the methodology used to identify a boundary lubricant in synovial fluid for cartilage-cartilage couples can be repeated using joint fluid and PE on Co-Cr. Many of the related references are given in section 2.1.2, but several more are given here; most of this work was conducted by Davis *et al.*,[11,12] David Swann,[13-18] and Gregory Jay.[19-25] Finally, since PE-on-metal is more conforming than metal-on-metal, it is worthwhile to consider whether lubricin may be the molecule that lubricates PE on Co-Cr. Rather than repeat the entire process of isolating this molecule, it would be worthwhile to simply assay joint fluid for lubricin. Unfortunately, no monoclonal antibody has yet been found for lubricin, so a biochemical assay for this protein is not yet known.

Gregory Jay has a latex-glass friction apparatus[21] that has been used to identify the presence of lubricin in synovial fluid. To examine whether lubricin is the essential joint fluid lubricant for PE on Co-Cr, bovine serum and joint fluid samples were sent to Jay's laboratory in Rhode Island for use in this apparatus. The results of these experiments are summarized below in Table 7.5.1. These results show that Jay's assay ranks lubricants differently than the apparatus in Chapter 5. In particular, bovine serum increased friction relative to saline lubrication. Furthermore, joint fluid samples that lubricated PE on Co-Cr well lubricated latex-on-glass poorly, and vice versa. These findings suggest that the essential boundary lubricant for PE on Co-Cr in joint fluid is *not* lubricin.

Table 7.5.1 Performance of selected lubricants in Jay's latex-on-glass apparatus versus the friction assay of Chapter 5 Two joint fluid samples and bovine serum are evaluated as lubricants for latex-on-glass and PE on Co-Cr. All data are presented as mean ± standard deviation, except for Jay's results, which are single values only. ID = Study ID. $\Delta\mu$ = difference in friction versus saline in Jay's apparatus. In these results, improved lubrication is measured by a reduced coefficient of friction (*i.e.*, $\Delta\mu < 0$). An explanation of the methodology for determining this value is found in Jay's published works given at the end of this chapter.

ID	*Description*	$\Delta\mu$	μ_s	μ_d
018	Primary TKA	- 0.0126	0.085 ± 0.008	0.046 ± 0.004
	Bovine serum	+ 0.0342	0.12 ± 0.02	0.054 ± 0.006
H16	Primary TKA	- 0.0512	0.12 ± 0.02	0.053 ± 0.004
H18	Primary TKA	-0.1078	0.16 ± 0.02	0.088 ± 0.008

7.5.2 Expand on Friction Studies

The friction studies I have performed are best described as preliminary. In comparison to the rheological study, which employed close to one hundred joint fluid samples, this ten sample study was miniscule. It would be useful to expand this study to confirm the variability and relationships described herein. Fortunately, the assay is relatively rapid, so such an expansion is possible.

This assay, while simple, does have its shortcomings. As described in Chapter 5, truncation of high forces prevented a more complete analysis of the relationship between normal load and friction, for example. Oscillatory results, even at steady-state, generated high variability in results, and may have confounded some comparisons. Finally, the device had to be calibrated constantly, and, as shown by the last experiments employing protein digestion, the friction measured on the device may have changed over time even when the calibration did not change. There is ample room for improvement to this assay.

A more sophisticated device was used to examine a small number of lubricants and surfaces in an additional pilot study. Using an AR2000 controlled stress rheometer (TA Instruments, New Castle, DE), the articulation of stainless steel on roughened copper was compared to the articulation of stainless steel on PE using a high molecular weight and concentration HA sample and bovine serum as lubricants. Using this apparatus, the frictional force was defined by the average force required to maintain a given velocity. The coefficient of friction was defined, then, by the ratio of frictional force to the applied normal load.

In these experiments, bovine serum was the same as that used in Chapter 5, and HA was a gift from Seikagaku Corporation (Tokyo, Japan). It was specified as 2.2 MDa and 10 mg/ml, much higher in concentration than the HA in joint fluid. This sample was used as received, and no analysis was performed to confirm its composition. Its viscosity was clearly greater than that of any joint fluid sample. It was chosen to find the effect of viscosity in this apparatus.

The roughness of the copper specimen was approximately 1 μm, and the roughness of the PE specimen was unknown. For both articulations, the top surface was a 20 mm diameter flat steel pin. The bottom surface was a thin disk (~ 10 mm) of much larger diameter. In each case, the surfaces were leveled using melted wax to form a deformable base for the bottom surface. Normal loads of 5 to 15 N were applied to reduce the nominal gap between the surfaces to zero. Shear force was measured at steady-shear through a wide range of shear rates, typically beginning at high shear rate. Shear force was divided by normal load to obtain a coefficient of friction. As necessary, experiments were repeated at different normal loads and shear rates to expand the range of measurement. The results of these experiments are given below in Figures 7.5.1 and 7.5.2.

At all velocities examined, HA led to a lower coefficient of friction in metal-on-metal than bovine serum did (Fig 7.5.1). In the metal-on-metal couple, it is easy to see a transition between boundary and fluid film lubrication for both lubricants. This articulation generates a Stribeck diagram similar to the one shown in Fig. 2.1.1. Thus, comparisons can be made between the lubricants in each lubrication regime. In boundary lubrication, the coefficient of friction between the surfaces is fairly similar, and appears to asymptotically approach the same value. HA led to transition to mixed and then to

fluid film lubrication at lower velocities. It is important to note, however, that different normal loads were employed in these experiments because different loads were required to reduce the gap between surfaces under different lubricants. Thus, analysis of F_f would have yielded different results than the present analysis of coefficient of friction. Furthermore, the different experimental conditions confound a direct comparison between lubricants.

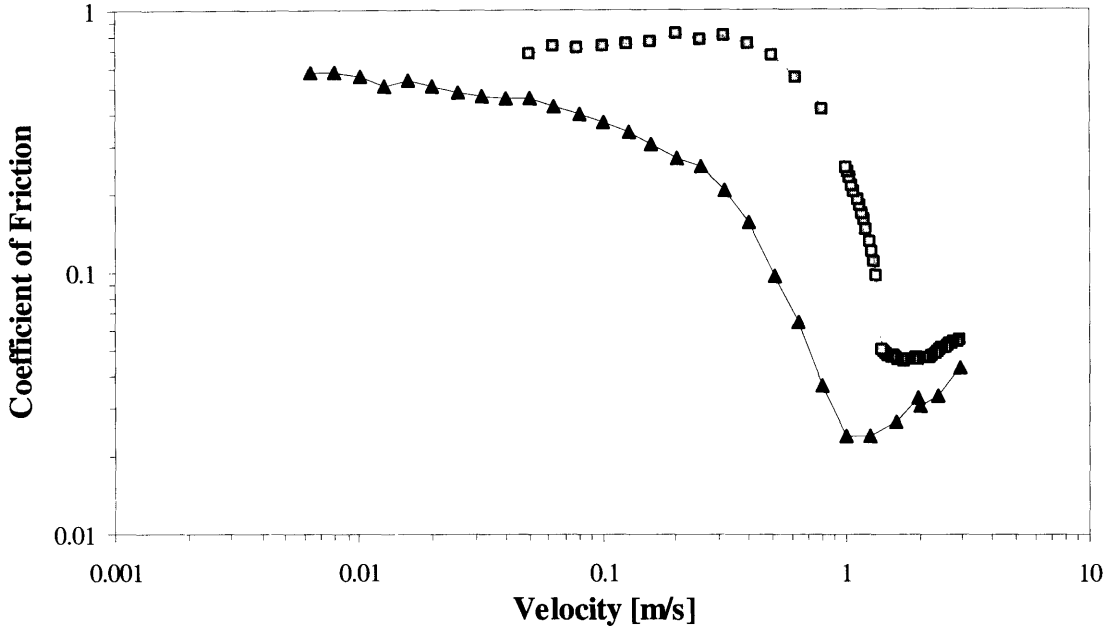

Fig. 7.5.1 Coefficient of friction versus velocity for stainless steel on copper using two different lubricants Gray squares represent bovine serum lubrication under 5 N normal load, and black triangles represent HA lubrication under 15 N normal load. The velocity used represents the linear velocity of the outer rim of the steel fixture. The curves demonstrate transition from boundary lubrication to fluid film lubrication with an intermediate regime of mixed lubrication.

In the metal-on-PE case (Fig. 7.5.2), no transition was observed from boundary to fluid film lubrication. One possible interpretation of this result is that the PE surface was not fully aligned with the steel surface. Specifically, when a load was applied to align two metal surfaces, the metal surfaces deformed very little, and most of the load was applied toward realigning the copper disk. In the metal-on-PE case, however, PE may have deformed to bear the load. Thus, less force was dedicated toward aligning the PE surface. Then a small part of the PE surface may have been in contact with the metal surface, causing the majority of friction. Such geometry would not favor a transition to fluid film lubrication. This explanation is not likely, however, since we were careful to examine the PE-metal interface during alignment, and alignment appeared to perfect to the eye. More likely, topographical differences between PA and copper led to this difference. Future tests on other PE specimens can elucidate the cause of this difference.

Lack of transition notwithstanding, friction was reduced between these surfaces at all velocities with HA as lubricant versus bovine serum. This result is consistent with the finding of metal-on-metal articulation. This finding is inconsistent, however, with the result in Chapter 5 that bovine serum reduced friction relative to HA in PBS. There are several difficulties in comparing the two systems, however. Loading conditions and

geometry are very different in the two cases, so one would not expect coefficient of friction to be comparable. In particular, at low loads, the assay of Chapter 5 did not distinguish among the lubricants. Nonetheless, one would not expect the *ranking* of lubricants to be different under different geometries. This is not a case, however, of comparison of like lubricants under different loading conditions. The HA used in the former experiments was physiologically relevant, whereas the present HA is much more concentrated, and the base solution is not known. The HA used in this experiment appears to reduce coefficient of friction between the surfaces, but may do so solely by its thickness. Even a single layer may behave more like a fluid film than a boundary lubricant if that layer is a network of interlocked macromolecules. Thus, a meaningful comparison between these results and those of Chapter 5 is not possible.

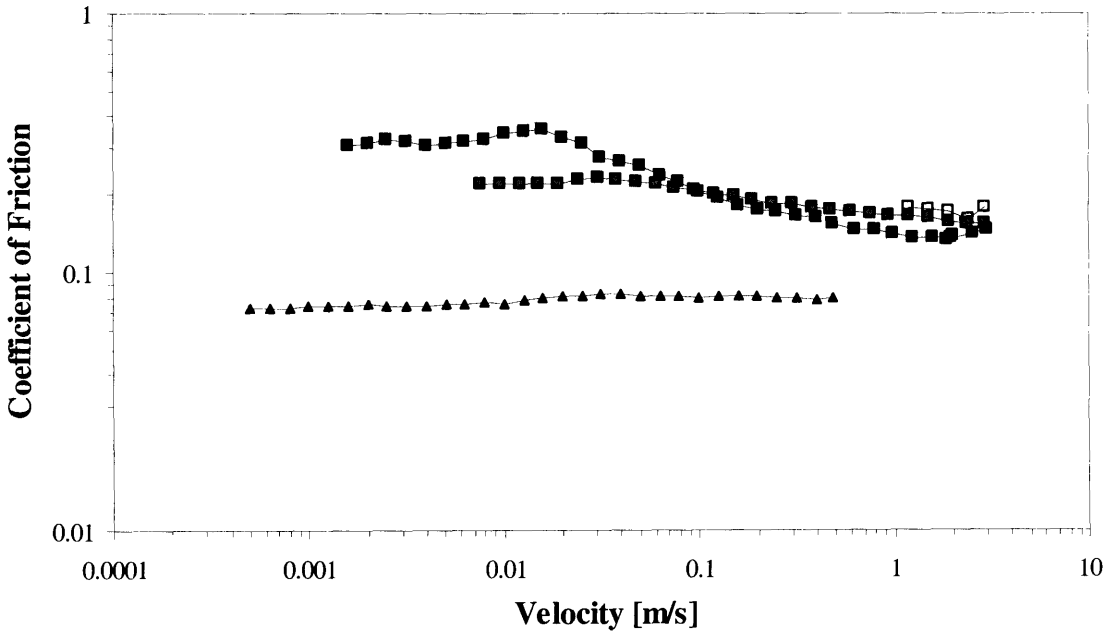

Fig. 7.5.2 Coefficient of friction versus velocity for stainless steel on PE using two different lubricants Squares represent bovine serum lubrication and triangles represent HA lubrication. Black shapes represent 15 N normal load, gray shapes represent 10 N normal load, and open shapes represent 5 N normal load. The velocity used represents the linear velocity of the outer rim of the steel fixture. No transition from boundary lubrication to fluid film lubrication is seen within the velocities tested.

A noteworthy difference between the curves in this chapter and the Stribeck curve (Fig. 2.1.1) is that the Stribeck curve traditionally normalizes the velocity by viscosity and normal load. (Normalization can be performed using other parameters, including a length scale, to generate a dimensionless load-rate abscissa.) There are several reasons not to include such curves presently, and they are generally related to the fact that characteristic parameters are difficult to pinpoint. Certainly, load is clearly defined, and one can mentally shift data gathered at higher load to the left in Figs. 7.5.1 and 7.5.2. Additionally, a length scale associated with the gap between surfaces is necessary to make the abscissa dimensionless. A characteristic length in this direction is often obtained using roughness parameter of the surfaces. This could be used to shift the steel-on-copper data relative to the steel-on-PE data. Vastly overshadowing these shifts is the viscosity shift, however. Using bulk viscosity, bovine serum has as viscosity of about

2 mPa s, whereas the viscosity of this HA sample was approximately 100 Pa s, more than four orders of magnitude greater than that of bovine serum.

Taking these values, we would find the HA curves shifted several orders of magnitude to the right of the bovine serum curves, even though this process is supposed to collapse the curves onto a master curve. The reasons for this difficulty are related to the use of characteristic parameters. First, the bulk viscosity may not represent the effective viscosity under the conditions of small gap, particularly in mixed lubrication. Second, the velocity used does not represent the true relative velocity of the surfaces. In disk on disk rotation, velocity increases from zero at the axis of rotation to the maximum value on the rim of the steel pin. An improved geometry is a hollow ring on a disk, in which the linear speed is almost identical at all points of contact. Changes to the geometry are currently being explored for this tribological apparatus. Likewise, small gap experiments could determine a viscosity more appropriate for this analysis.

Having worked out these details, this device could be useful both for small gap rheology and for tribology. For example, the effect of normal load, changes in gap between the surfaces, and the effect of surface roughness could all be examined in detail more easily on this device than on the device of Chapter 5. Specifically, this device requires less calibration, can vary and measure load and velocity continuously, and calculates mean values in steady-shear more easily than the former apparatus. The major limitation of this device is that it is not approved for use with biological fluids, and therefore cannot be used to measure real joint fluid samples.

With regard to moving forward with joint fluid friction experiments, the most important experiments to perform are more measurements of friction in PE on Co-Cr using joint fluid samples as lubricants. I would recommend modifying joint fluid: separate it into aliquots using the same biochemical techniques used to isolate lubricin; and measure friction using each aliquot. In this fashion, the boundary lubricating molecule(s) in joint fluid may be identified.

7.5.3 Quantify the Illustration

A third direction in which this research could move would be to quantify this schematic illustration of boundary lubrication and wear in TJA. This may take two forms. One direction is an examination of the adherence of components of joint fluid to metals and PE. For example, surface analytical techniques such as atomic force microscopy can measure repulsive and adhesive forces of various molecules using a PE surface and metal stylus, or vice versa. Other techniques can be used to determine which molecules in joint fluid preferentially adhere to metals, enabling boundary lubrication. In this way, lubricants and surfaces can be compared in a quantitatively useful manner.

A separate approach to quantifying this model would be to perform finite element analysis on THA and TKA using the three regime description of PE wear given in section 6.5. Such a study would begin by verifying the model using parameters estimated from the literature. Then, by modifying material properties and geometry of the articulation, one could predict what would be required to create a prosthesis that encounters insignificant wear.

7.5.4 Design New THA and TKA

Finally, each of these topics touches on the ultimate goal of TJA research, a better prosthesis. Results from each area of research can contribute to a better choice of materials, an improved geometry, or some other change to improve the performance of joint prostheses. The ultimate solution to prosthesis failure in TJA will most likely come from an improved prosthesis design, though the ideas and principles behind the design will come through research such as that presented in this thesis.

7.5.5 Additional Studies

There are a number of additional studies that would be useful to perform, but these are lower priorities than the above topics. These are listed below:

- Expansion of wear tests to include a greater range of loads and stresses, preferably extending into all three wear regimes
- Correlation of friction tests to wear tests using HA, protein, and phospholipid as lubricants
- Measurement of composition, properties, and friction of joint fluid from individual patients in a longitudinal fashion, as well as from successful arthroplasty (*i.e.*, obtained at autopsy)
- Histological analysis of the synovial membrane to explain the inverse correlation between synthesized and filtered components of joint fluid

7.6 Closure

In this thesis, I have shown that joint fluid is a principal determinant of the tribology of Co-Cr on PE prostheses. I showed how joint fluid flow properties and composition vary. I explored the effects of individual components on the tribology of PE on Co-Cr, and showed the presence of a boundary lubricating molecule other than HA, albumin, γ-globulin, or DPPC. I also showed how contact area may be a dominant geometrical determinant of wear in this articulation, and how load may actually have little relevance within a certain range. Finally, I developed a schematic model for understanding these results in terms of real contact between the surfaces. And that is all I have to say about that.

7.7 References

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APPENDICES

Appendix A: Patient Summaries

Study ID #	Patient ID #	Notebook Page	Gender	Age	Left/Right	Indication	Date Obtained	Height (in)	Weight (lb)	Occasion	Surgeon
003	01596634	127-94	Female	66	Left	Wear-related failure	6/26/2000	NR	NR	Revision	Estok
005	08995615	N6-8	Male	81	Right	Osteoarthritis	6/30/2000	NR	NR	TKA	Brick
006	15889520	127-94,96,99	Female	86	Right	Osteoarthritis	6/20/2000	NR	NR	TKA	Wright
007	12945044	140-05,8, N9	Female	83	Left	Osteoarthritis	6/30/2000	NR	NR	TKA	Wright
008	14789499	140-05	Female	68	Right	NR	7/10/2000	NR	NR	TKA	Scott
009	M0694412	140-06,8,N11	Male	76	Left	Osteoarthritis	7/11/2000	NR	NR	TKA	Rubash
010	14728596	140-10, N19	Female	70	Left	Osteoarthritis	7/17/2000	NR	NR	TKA	Scott
011	08226987	140-10, N15	Female	71	Left	Osteoarthritis	7/18/2000	NR	NR	TKA	Brick
012	011B	140-11, N17	NR	NR	NR	Effusion	12/29/1999	NR	NR	Effusion	Fitz
013	010B	140-11, N17	NR	NR	NR	Effusion	12/29/1999	NR	NR	Effusion	Fitz
014	012B	140-11	NR	NR	NR	Effusion	12/29/1999	NR	NR	Effusion	Fitz
015	013B	140-11, N20	NR	NR	NR	Effusion	12/29/1999	NR	NR	Effusion	Fitz
016	014B	140-11	NR	NR	NR	Effusion	12/29/1999	NR	NR	Effusion	Fitz
017	003B	140-21	NR	NR	NR	Effusion	12/29/1999	NR	NR	Effusion	Fitz
018	05415195	140-14, N25,29	Male	68	Right	Osteoarthritis	4/7/2000	NR	NR	TKA	Wright
019	03287315	140-21, N21,27	Male	72	Left	Wear, Osteolysis	7/31/2000	NR	NR	Revision	Scott
020	09090655	140-33	Male	63	Right	Osteoarthritis	8/14/2000	NR	NR	TKA	Miegel
021	11887932	None	Female	64	Left	Osteoarthritis	11/18/1999	NR	NR	TKA	Wright
022	08867020	140-37	Female	59	Right	Osteoarthritis	8/18/2000	NR	NR	TKA	Estok
023	12227872	140-53	Male	72	Right	Failed TKA (septic)	9/6/2000	NR	NR	Revision	Brick
024	03850294	140-53, N29	Female	72	Right	Osteoarthritis	9/11/2000	NR	NR	TKA	Estok
025	08243123	140-52, N23-5	Female	70	Right	Osteoarthritis	9/11/2000	NR	NR	TKA	Estok
026	11692357	140-55	Female	74	Left	Osteoarthritis	9/15/2000	NR	NR	TKA	Scott
027	09102583	140-55	Male	79	Left	Osteoarthritis	9/18/2000	NR	NR	TKA	Scott
028	03032521	140-55	Female	79	Left	Osteoarthritis	9/19/2000	NR	NR	TKA	Scott
029	N0992554	140-57, N27-8	Female	73	NR	Osteoarthritis	9/19/2000	NR	NR	TKA	Scott
030	N1019289	140-57	Male	69	Left	Effusion after TKA	9/19/2000	NR	NR	TKA-E	Scott
031	03276011	140-60,79, N36	Female	65	Left	Osteoarthritis	9/25/2000	NR	NR	TKA	Thornhill
032	15373699	140-60	Female	85	Left	Osteoarthritis	9/25/2000	NR	NR	TKA	Minor
033	16041972	140-62, N35	Female	88	Right	Osteoarthritis	9/22/2000	NR	NR	TKA	Brick
034	N0993822	140-62, N31	Male	64	Right	Osteoarthritis	10/3/2000	NR	NR	TKA	Scott
035	16245045	140-62, N34	Male	78	Right	Osteoarthritis	10/2/2000	NR	NR	TKA	Thornhill
036	N163782	140-62	Female	68	Left	Osteoarthritis	10/4/2000	NR	NR	TKA	Scott
037	N0992553	140-62,80	Female	50	Right	Osteoarthritis	10/3/2000	NR	NR	TKA	Scott
038	01543214	140-63	Female	73	Right	Osteoarthritis	9/29/2000	NR	NR	TKA	Wright
039	16307514	140-63	Female	72	Right	Osteoarthritis	10/2/2000	NR	NR	TKA	Scott
040	16405367	140-63	Female	70	Right	Osteoarthritis	10/2/2000	NR	NR	TKA	Scott

NR = Not reported or not recorded

TKA-E = Effusion after TKA

Appendix A: Patient Summaries

Study ID #	Patient ID #	Notebook Page	Gender	Age	Left/Right	Indication	Date Obtained	Height (in)	Weight (lb)	Occasion	Surgeon
169	N1094422	153-67	Male	79	Right	Wear, Osteolysis	7/31/2001	NR	NR	Revision	Scott
170	N1095852	153-68	Female	75	Left	Unstable TKA	8/1/2001	NR	NR	Revision	Scott
171	N280542	153-91	Male	75	Right	Pre-Revision	8/22/2001	NR	NR	TKA-E	Scott
172	14053912	4-11	Female	42	Right	Wear (Post-Traumatic)	10/12/2001	NR	NR	Revision	Wright
173	N1118819	4-21	Male	69	NR	Painful, stiff TKA	10/23/2001	NR	NR	Revision	Scott
174	N1080502	4-21	Female	48	NR	Lupus, Loose TKA	10/30/2001	NR	NR	Revision	Scott
175	N0005426	4-34	Male	NR	Left	Mechanical (not wear)	11/20/2001	NR	NR	Revision	Scott
176	J.McCormack	4-63	Male	NR	NR	Effusion	3/19/2002	NR	NR	TKA-E	Scott
177	L.Marshall	4-72	Female	NR	NR	Wear, Osteolysis	4/10/2002	NR	NR	Revision	Scott
178	N1035670	4-94	Female	72	NR	Wear, Osteolysis	6/4/2002	NR	NR	Revision	Scott
6*	NR	None	NR	NR	NR	NR	NR	NR	NR	TKA	NR
B	NR	None	NR	NR	NR	NR	NR	NR	NR	TKA	NR
H01	386-68-8680	153-99	Male	43	Left	Osteoarthritis	9/24/2001	70	219	TKA	Schaefer
H02	381-28-0769	4-07	Male	77	Right	Osteoarthritis	10/1/2001	71	172	TKA	Schaefer
H03	382-64-4680	4-21	Female	45	Right	Osteoarthritis	10/8/2001	67	200	TKA	Schaefer
H04	376-46-7259	4-32	Female	56	Right	Osteoarthritis	11/5/2001	68	210	TKA	Schaefer
H05	316-50-2505	4-34	Female	38	Right	Osteoarthritis	11/13/2001	63	213	TKA	Schaefer
H06	472-44-2435	4-34	Female	58	Left	Osteoarthritis	11/12/2001	66	230	TKA	Schaefer
H07	1520913-3	4-41	Male	68	Right	Osteoarthritis	12/4/2001	70	198	TKA	Schaefer
H08	1520913-3	4-41	Male	68	Left	Osteoarthritis	12/4/2001	70	198	TKA	Schaefer
H09	382-64-4680	4-63	Female	45	Left	Osteoarthritis	2/18/2002	67	200	TKA	Schaefer
H11	373-44-6323	4-55	Male	60	Left	Osteoarthritis	1/7/2002	71	216	TKA	Schaefer
H12	386-28-7380	4-55	Female	70	Right	Osteoarthritis	1/21/2002	59	145	TKA	Schaefer
H13	383-68-2327	4-55	Male	42	Right	Osteoarthritis	1/14/2002	75	240	TKA	Schaefer
H14	369-60-9041	4-55	Male	45	Right	Osteoarthritis	1/14/2002	71	290	TKA	Schaefer
H15	368-16-6155	4-55	Male	80	Left	Osteoarthritis	12/10/2001	67	187	TKA	Schaefer
H16	00152246	4-73	NR	NR	NR	NR	NR	NR	NR	TKA	Schaefer
H17	292-26-7606	4-72	Male	68	Left	Osteoarthritis	3/18/2002	68	NR	TKA	Schaefer
H18	1545403-6	4-72	Male	75	Left	Osteoarthritis	3/18/2002	70	181	TKA	Schaefer
H19	367-40-7198	4-72	Male	60	Left	Osteoarthritis	3/19/2002	73	225	TKA	Schaefer
H20	370360382	4-72	Male	52	Left	Post-Traumatic Arthritis	3/19/2002	67	186	TKA	Schaefer
H21	386-28-7380	4-73	Female	70	Left	Osteoarthritis	3/25/2002	60	143	TKA	Schaefer
H22	270-50-0618	4-73	NR	NR	NR	NR	NR	NR	NR	TKA	Schaefer
H23	288-32-5376	4-94	Female	66	Right	Osteoarthritis	~5/29/02	62	150	TKA	Schaefer
NR	00032601	4-72	NR	NR	NR	NR	NR	NR	NR	Effusion	Lynch
NR	372-20-4814	None	Female	76	Right	Osteoarthritis	4/15/2002	63	NR	TKA	Schaefer
NR	367-40-7198	None	Male	60	Right	Osteoarthritis	5/16/2002	73	216	TKA	Schaefer

NR = Not reported or not recorded

TKA-E = Effusion after TKA

Appendix A: Patient Summaries

Study ID #	Patient ID #	Notebook Page	Gender	Age	Left/Right	Indication	Date Obtained	Height (in)	Weight (lb)	Occasion	Surgeon
041	N0995452	140-63	Female	72	Right	Osteoarthritis	10/4/2000	NR	NR	TKA	Scott
042	N0996695	140-63, N33	Female	78	Right	Osteoarthritis	10/10/2000	NR	NR	TKA	Scott
043	N0997947	140-63	Male	72	Right	Osteoarthritis	10/10/2000	NR	NR	TKA	Scott
044	N0997614	140-63, N32	Female	83	Right	Osteoarthritis	10/11/2000	NR	NR	TKA	Scott
045	N0994806	140-63, N32	Male	63	Left	Osteoarthritis	10/11/2000	NR	NR	TKA	Scott
046	M*R**Z*	N36	Unknown	NR	Unknown	NR	NR	NR	NR	TKA	NR
047	N1000456	N33	Female	70	Left	Unstable TKA	10/25/2000	NR	NR	Revision	Scott
048	013B2	N20	Unknown	NR	Unknown	Effusion	12/29/1999	NR	NR	Effusion	Fitz
050	NR	NR	NR	NR	NR	NR	NR	NR	NR	TKA	Scott
141	N0991930	140-80	Female	78	Left	Osteoarthritis	10/24/2000	NR	NR	TKA	Scott
142	N1002411	140-80	Female	88	Left	Osteoarthritis	10/24/2000	NR	NR	TKA	Scott
143	N1001538	140-80	Female	89	Right	Osteoarthritis	10/18/2000	NR	NR	TKA	Scott
144	14922884	140-81	Female	58	Right	Osteoarthritis	10/30/2000	NR	NR	TKA	Scott
145	N1001538	140-82	Female	89	Left	Osteoarthritis	10/18/2000	NR	NR	TKA	Scott
146	N1001539	140-83	Female	73	Left	Osteoarthritis	10/18/2000	NR	NR	TKA	Scott
147	N0997930	140-84	Female	68	Right	Osteoarthritis	10/24/2000	NR	NR	TKA	Scott
148	N1001539	140-84	Female	73	Right	Osteoarthritis	10/18/2000	NR	NR	TKA	Scott
149	N1006466	140-84	Female	66	Right	Osteoarthritis	11/7/2000	NR	NR	TKA	Scott
150	N0997931	140-84	Female	62	Left	Osteoarthritis	10/17/2000	NR	NR	TKA	Scott
151	N1008223	140-84	Male	68	Left	Osteoarthritis	11/7/2000	NR	NR	TKA	Scott
152	N1003864	140-85	Female	52	Right	Osteoarthritis	11/1/2000	NR	NR	TKA	Scott
153	N1003864	140-85	Female	52	Left	Osteoarthritis	11/1/2000	NR	NR	TKA	Scott
154	12451431	140-86	Male	64	Right	Osteoarthritis	10/26/2000	NR	NR	TKA	Poss
155	N1001713	140-87	Female	68	Right	Osteoarthritis	10/31/2000	NR	NR	TKA	Scott
156	N1001713	140-87	Female	68	Left	Osteoarthritis	10/31/2000	NR	NR	TKA	Scott
157	16383325	N/A	Female	77	Left	Osteoarthritis	11/6/2000	NR	NR	TKA	Scott
158	16445546	140-88	Male	54	Right	Osteoarthritis	11/6/2000	NR	NR	TKA	Scott
159	04696498	140-88	Female	71	Right	Osteoarthritis	11/6/2000	NR	NR	TKA	Scott
160	N1013637	140-87	Female	61	Right	Wear, Osteolysis	11/29/2000	NR	NR	Revision	Scott
161	N1013123	140-87	Female	69	Right	Synovitis, wear	11/28/2000	NR	NR	Revision	Scott
162	16559635	140-88	Male	73	Left	Wear, Osteolysis	12/4/2000	NR	NR	Revision	Scott
163	N1021180	153-05	Female	76	Left	Unstable TKA	12/20/2000	NR	NR	Revision	Scott
164	N1019289	153-05	Male	69	Left	Polyethylene Wear	12/19/2000	NR	NR	Revision	Scott
165	N1035169	153-14	Female	67	Left	Resurfacing, exchange	2/6/2001	NR	NR	Revision	Scott
166	N1052907	153-30	Male	37	Left	Polyethylene Wear	3/28/2001	NR	NR	Revision	Scott
167	N1050900	153-38	Male	71	Left	Worn uni TKA	3/20/2001	NR	NR	Uni Rev	Scott
168	N1059782	153-39	Female	63	NR	Wear-related failure	4/20/2001	NR	NR	Revision	Scott

NR = Not reported or not recorded

TKA-E = Effusion after TKA

APPENDIX B
AN ALTERNATE RHEOMETER CALIBRATION SCHEME

Using a constant to relate the expected shear rate to the measured shear rate did not eliminate the entire difference between the two curves. In particular, at low stress, the ratio of the measured to expected viscosity was larger than at high shear stress (as shown in the logarithmic plot of Fig 3.4.1. This finding occurred frequently in calibration, and was probably related to the 0.5 Pa minimum placed on shear stress by the manufacturers. This finding persisted even after calibration of bearing friction correction – a possible cause was eccentricity between the cylinder and rotor. Whatever the cause, this tendency indicated that measurements at low shear stress overestimated fluid viscosity by a small amount, possibly resulting in overestimated η_0 and c, since shear-thinning could appear to continue at artificially low shear rates.

There were means to correct for this overestimation, such as a continuously varying calibration constant. For example, using a power law relationship instead of a Newtonian relationship for the measured shear rate of the viscosity standard, one can obtain $\dot{\gamma}(\tau) = A\tau^B$, where A and B are constants. Thus, instead of a constant C, as calculated above, one could have multiplied each shear rate by $C(\tau)$, the ratio of the actual standard viscosity to the calculated standard viscosity as a function of shear stress. Using a power law fit for the calibration curve shown above, we would have had

$$C(\tau) = \frac{\dot{\gamma}_{Expected}}{\dot{\gamma}_{Measured}} = \frac{9.852\tau}{6.954\tau^{1.0414}} = 1.417\tau^{-0.0414},$$

where the units have been removed for simplicity.

Fig. B.1 (below) shows the is the viscosity versus shear rate curve for a sample of synovial fluid obtained at primary TKA whose viscosity was measured immediately following the calibration discussed above. Curves are shown for the raw data as well as the data calibrated using a linear fit (constant C) and a power law fit. These data have been fit to the Cross model, and the parameters are listed in Table B.1 (below). When these data are fit to curves, as has been done for comparison, one sees that using different schemes to calibrate the measurement produces different results. Both c and η_0 vary substantially when calibrated using a power law rather than linear regression, reflecting the relative importance of the low shear rate values in determining these parameters. Rate index and η_{1Pa}, on the other hand, are affected much less by the means of calibration.

Throughout the experiments, calibration has been performed by linear regression. More complex means of calibration, such as the one described above, have been ignored, with the expectation that even a 20% maximum difference in η_0 and 41% maximum difference in c will not affect the statistical significance of results in groups in which these parameters vary over several orders of magnitude.

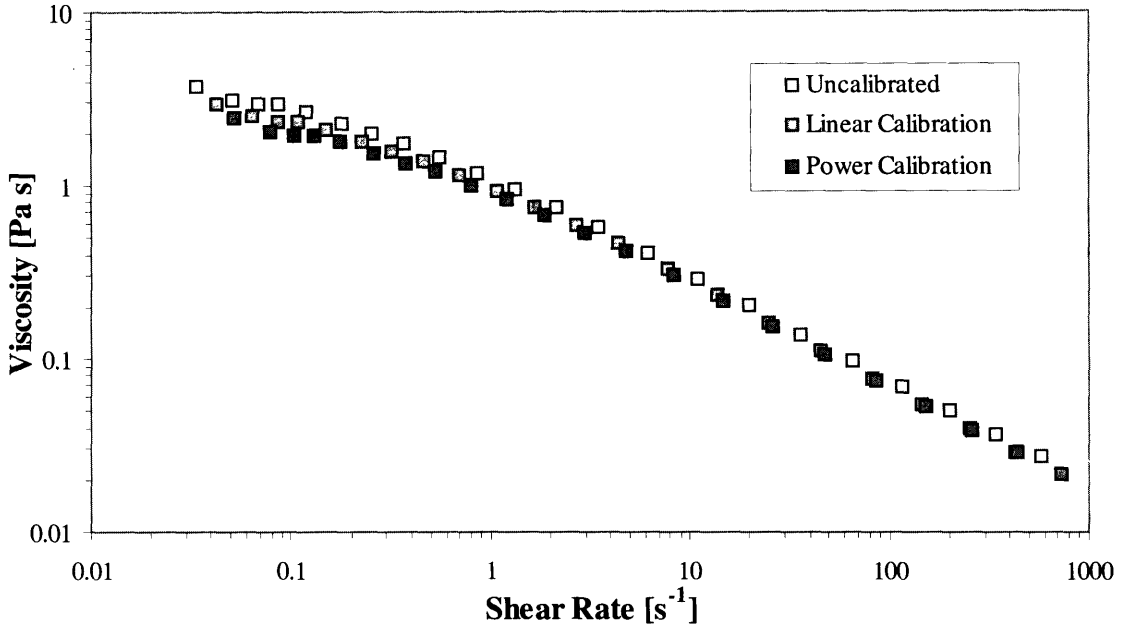


Fig B.1 and Table B.1 Comparison of results using three different calibration schemes, fit to the Cross model Open squares were not calibrated, light squares were calibrated using a constant ratio, and dark gray squares were calibrated using a power law fit. Synovial fluid sample obtained at index TKA from a 60 year old man with OA (Study ID H11). There is very little difference between the results except at very low shear rates. Even though the data points are not much different, the parameters of the Cross model are affected by what means are used to process the values obtained at low shear rates.

Parameter	*Means of Calibration*		
	None	*Linear Regression*	*Power Law*
η_{1Pa} (Pa s)	1.2	0.92	0.82
η_0 (Pa s)	6.3	5.0	4.0
ln η_0	1.84	1.61	1.39
c (s)	14	11	7.8
d	0.61	0.61	0.61

APPENDIX C
f_C AND G_C CALCULATED

Calculation of f_c was performed by interpolation between the two frequencies nearest the crossover of the two moduli. The highest frequency at which $G' < G''$ was labeled f_1 and the lowest frequency at which $G' > G''$ was labeled f_2. Moduli at each frequency were labeled with appropriate subscripts. Crossover was determined from the equation

$$f_c = \frac{f_2 \cdot (G_1'' - G_1') + f_1 \cdot (G_2' - G_2')}{G_1'' - G_1' + G_2' - G_2''}.$$

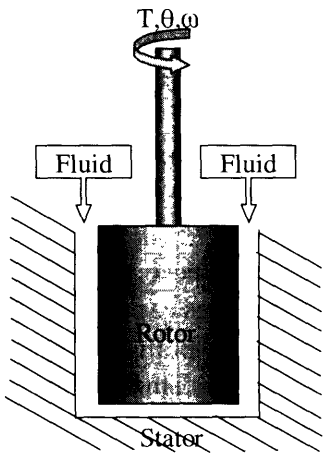
Modulus at crossover was determined from the equation

$$G_c = \frac{G_2'' \cdot (f_c - f_1) + G_1'' \cdot (f_2 - f_c)}{f_2 - f_1}.$$

These calculations assume that both moduli vary linearly between f_1 and f_2.

APPENDIX D
SHEAR STRESS CALCULATED FROM OSCILLATORY TORQUE

It was necessary to calculate the shear stress applied to the joint fluid sample when a given oscillatory torque was applied. Using the figure to the right as a guide, a sample calculation is given here. The figure schematically represents the double cylinder Couette cell, with a stator and a rotor. The actual device includes an inner and an outer stator, such that the inner and outer surfaces of the rotor both apply shear to the fluid.



As an example, I calculate the shear stress applied to the fluid when 25 μNm of torque is applied to the rotor. This torque, T, is related to the shear stress on the fluid by the equation $T = \int R\sigma\, \delta A$, where R is the radius on which the torque acts, σ is the shear stress, and δA is the surface area of the rotor (and the area of integration). For the geometry of the Couette cell, there are two radii. R_1 is 20.70 mm (inner rotor surface) and R_2 is 21.00 mm (outer rotor surface), and the surface area of each is $2\pi RH$, where H is the height of the rotor (20.50 mm).

We assume that the shear stress applied to the fluid is the same on both surfaces. This assumption is similar to the assumption that shear stress is constant throughout the fluid, and is true when $(R_2 - R_1)/R_1 \ll 1$. Since 0.70 mm/20.70 mm = 0.03, this assumption is valid.

Thus, $T = \sigma\, 2\pi H\left(R_1^2 + R_2^2\right)$; rearranging, $\sigma = T/\left(2\pi H\left(R_1^2 + R_2^2\right)\right)$. Using the values for the geometry of this particular Couette cell, $\sigma = 0.223$ Pa corresponds to 25 μNm.

APPENDIX E
VALIDATION OF RHELOGIGAL METHODS

The experimental protocol described in Chapter 3 was validated during the process of gathering data. Many samples were obtained with small amounts of fluid; it was essential to preserve joint fluid to allow for other types of analysis. Consequently, rheological experiments could not be performed on triplicate or even duplicate aliquots. Therefore, the repeatability of experiments was verified in a few samples only.

E.1 Duplication of the Experiment & Dependence on Deformation History

One measure of the reliability of an experiment is its repeatability, whether the same experiment on the same sample reveals the same values in consecutive measurements. There has been some question as to whether this is the case in normal synovial fluid. In particular, a number of authors have found thixotropic behavior in synovial fluid, and care must be taken to account for this effect when measuring steady-shear viscosity.

Consequently, one sample has been evaluated using a protocol adapted from Oates, *et al.*[1] For this sample, the second decade of data points (from 1 Pa to 0.1 Pa) was determined using the average of eighty seconds of shearing for each point, without waiting for equilibrium. After the decade was completed, the decade was repeated. Thixotropic behavior would manifest itself in higher viscosity in the repeated decade. In order to ensure that any difference was due to a fluid change reversible by shearing, a third run of the same decade was performed after 5 minutes of shearing at 500 s^{-1} to erase previous shear history. The results of these three runs are shown below in Fig. E.1. The parameters determined from fitting the results to the Cross model are given below in Table E.1.

These results demonstrate the repeatability of the measurement, in that the same shear stress applied to the same sample brings about the same shear rate. Rate index and η_0 were both obtained with less than 10% coefficient of variation,* but c was more variable. This finding suggests that c may be a poor parameter to for comparison, due to the high variability. These results do, however, justify a single measurement through the range of shear rates.

Furthermore, these results suggest that shear history does not affect steady-shear viscosity at low shear rates, though, as discussed in section 3.5.5, another sample appeared to be affected by deformation history. In any case, the use of ten seconds of preshear is justified.

* Calculation of coefficient of variation for η_0 is determined based on the natural logarithm of the value, not the value itself.

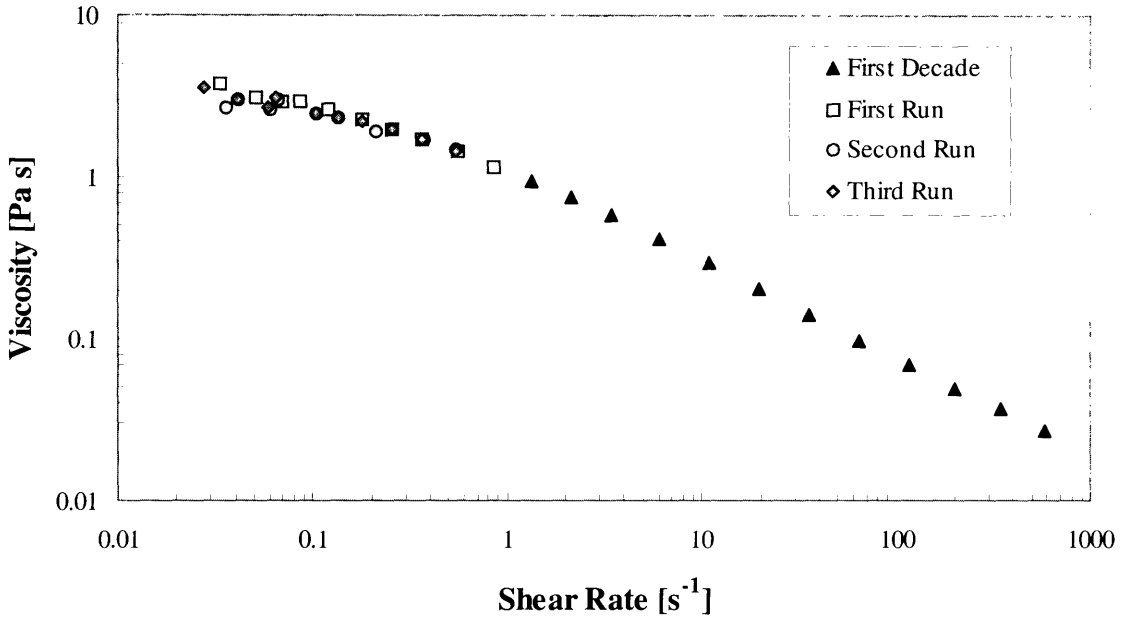


Figure E.1 and Table E.1 Examination of thixotropic behavior in synovial fluid This sample of synovial fluid obtained from a 45 year old woman at index TKA (Study ID H09) does not exhibit thixotropic behavior. Three repeated measures of the same sample resulted in very small differences between the measurements. Likewise, when fit to the Cross model, the parameters show little variability. Rate index shows particularly little variability, whereas consistency varies substantially.

	Run 1	*Run 2*	*Run 3*	*Mean ± Standard Deviation*
ln η_0 (Pa s)	1.7	1.4	1.5	1.5 ± 0.1
η_0 (Pa s)	5.3	4.1	4.6	4.7 ± 0.6
c (s)	9.1	5.0	6.6	6.9 ± 2.0
d	0.62	0.64	0.63	0.63 ± 0.01

E.2 Intra-Sample Repeatability

For several samples, the viscosity experiment was performed on separate aliquots within a sample to verify that successive aliquots brought about identical results. In such cases, after the viscosity and linear viscoelastic properties had been measured, the apparatus was washed and rinsed, and the viscosity protocol repeated on a second sample. One such sample is shown below in Figure E.2.

The real difference between these two measurements is somewhat obscured by the double logarithmic scale. Specifically, since ln $\eta_0 \sim 0$ for both samples, the variation of 0.4 between them makes the coefficient of variation large. Again in this case, c is quite variable, and d does not vary between the two aliquots.

Since it has already been shown that measuring the same sample twice does not cause this variability, there must be a difference between the aliquots. The most likely cause of this variability is sample non-homogeneity. Perhaps more viscous portions sample are less likely to be aspirated into the pipette for transfer to the rheometer in the first attempt. Later, when the second aliquot is taken, the viscosity of the remaining

sample is slightly higher due to this effect. Therefore, it is important to try to obtain a homogenous mixture of sample in each aliquot.

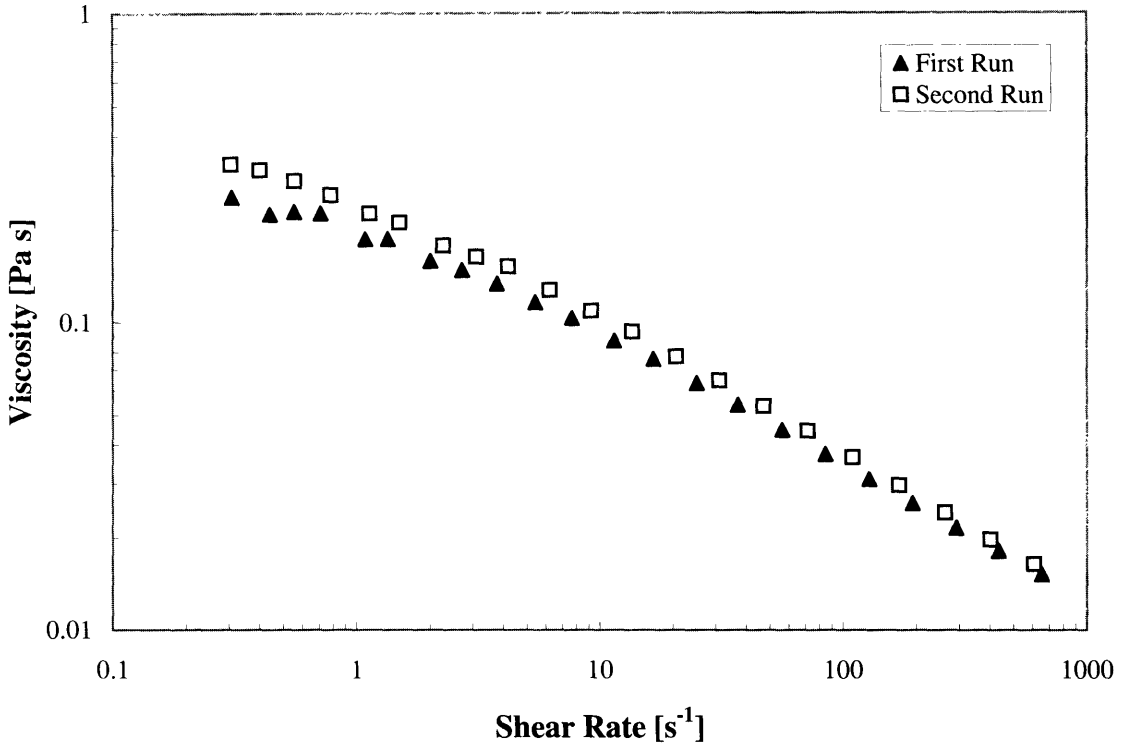


Figure E.2 and Table E.2 Examination of intra-sample variability in synovial fluid Squares and triangles represent two different aliquots of the same sample of synovial fluid obtained from a 60 year old man at index TKA (Study ID H11). The two samples exhibit slightly different rheological properties, possibly due to less viscous portions of the sample being preferentially pipetted in the first aliquot. Below, these data are fit to the Cross Model.

	Run 1	*Run 2*
ln η_0 (Pa s)	-0.84	-0.47
η_0 (Pa s)	0.43	0.62
c (s)	1.2	2.6
d	0.49	0.49

E.3 Reference

1. K. Oates, W. Krause and R. Colby: Using rheology to probe the mechanism of joint lubrication: polyelectolyte/protein interactions in synovial fluid. In: *Materials Research Society*, Boston, MA, 2001.

Appendix F: Description of Joint Fluid Samples

Study ID #	Occasion	+Quantity	Additional Gross Description	Blood ?	Gross Category	Location of remainder
003	Revision	22.0 mL	None	No	Non-inflammatory	3 MCF and 1 CF Tube
005	TKA	NR	None	No	Non-inflammatory	None Remains
006	TKA	7.0 mL	Slightly red, stringy	Yes	Hemarthrosis	3 MCF and 1 CF Tube
007	TKA	8.5 mL	None	No	Non-inflammatory	None Remains
008	TKA	2.0 mL	None	Yes	Bloody	None Remains
009	TKA	7.1 mL	None	No	Non-inflammatory	4 Microcentrifuge Tubes
010	TKA	6.0 mL	None	Yes	Bloody	None Remains
011	TKA	9.0 mL	Clear, yellow, normal consistency	No	Non-inflammatory	4 MCF and 1 CF Tube
012	Effusion	7.0 mL	May have been switched with #016	No	Non-inflammatory	4 Microcentrifuge Tubes
013	Effusion	6.5 mL	Pink	No	Hemarthrosis	1 Microcentrifuge Tube
014	Effusion	< 1 mL	None	No	NR	None Remains
015	Effusion	1.5 mL	pink	No	Hemarthrosis	None Remains
016	Effusion	7.0 mL	Watery, yellowish, some precipitates	No	Inflammatory	None Remains
017	Effusion	NR	None	No	NR	None Remains
018	TKA	19.0 mL	Yellow, with no particles	No	Non-inflammatory	4 MCF and 1 CF Tube
019	Revision	22.4 mL	Contained Blood	Yes	Bloody	3 Microcentrifuge Tubes
020	TKA	1.5 mL	Clear, but insufficient for viscometry	No	Non-inflammatory	None Remains
021	TKA	2.0 mL	Insufficient for viscometry	No	NR	None Remains
022	TKA	1.0 mL	Insufficient for viscometry	No	NR	None Remains
023	Revision	NR	None	No	NR	None Remains
024	TKA	2.5 mL	Some precipitate, yellow	No	Non-inflammatory	None Remains
025	TKA	11.5 mL	Yellow, with white particles	No	Non-inflammatory	4 Microcentrifuge Tubes
026	TKA	1.5 mL	Insufficient for viscometry	Yes	Bloody	None Remains
027	TKA	4.0 mL	Blood layer above yellow, viscous layer	Yes	Non-inflammatory	None Remains
028	TKA	3.0 mL	Pinkish, aggregations of RBC's present	Yes	Hemarthrosis	None Remains
029	TKA	6.0 mL	None	No	NR	None Remains
030	TKA-e	4.5 mL	None	No	Hemarthrosis	None Remains
031	TKA	14.0 mL	Some precipitate	No	Septic	2 MCF and 1 CF Tube
032	TKA	NR	Yellow, clear, chalky	No	Non-inflammatory	None Remains
033	TKA	3.0 mL	Orange-yellow, with precipitates	No	Hemarthrosis	None Remains
034	TKA	6.0 mL	Yellow, no precipitate	No	Non-inflammatory	None Remains
035	TKA	3.0 mL	Yellow, clear, some precipitates	No	Non-inflammatory	None Remains
036	TKA	NR	None	No	NR	None Remains
037	TKA	5.0 mL	Some precipitate	No	Septic	None Remains
038	TKA	NR	None	No	NR	None Remains
039	TKA	2.5 mL	None	No	NR	None Remains
040	TKA	9.0 mL	Some Precipitate	No	Septic	Microcentrifuge Tube

NR = Not reported or not recorded

+ Not all fluid from joint was recovered.

*Destroyed in cleanup, March, 2002

Appendix F: Description of Joint Fluid Samples

Study ID #	Occasion	+Quantity	Additional Gross Description	Blood ?	Gross Category	Location of remainder
041	TKA	1.5 mL	None	No	NR	None Remains
042	TKA	3.0 mL	Some precipitate	No	Septic	None Remains
043	TKA	1.5 mL	None	No	NR	None Remains
044	TKA	3.0 mL	Lots of precipitate	No	Septic	Microcentrifuge Tube
045	TKA	6.5 mL	Thick, but normal consistency	No	Inflammatory	None Remains
046	TKA	3.5 mL	None	No	Hemarthrosis	None Remains
047	Revision	2.0 mL	Watery, pink, and clear	No	Hemarthrosis	1 MCF and 1 CF Tube
048	Effusion	2.0 mL	Clear, yellow, normal consistency	No	Non-inflammatory	None Remains
050	TKA	NR	None	No	NR	2 Microcentrifuge Tubes
141	TKA	NR	None	No	NR	None Remains
142	TKA	6.0 mL	Very Viscous	No	Non-inflammatory	None Remains
143	TKA	4.0 mL	None	No	Non-inflammatory	None Remains
144	TKA	15.0 mL	Inviscid	No	Inflammatory	None Remains
145	TKA	7.0 mL	None	No	Non-inflammatory	None Remains
146	TKA	6.0 mL	Inviscid	No	Inflammatory	None Remains
147	TKA	6.5 mL	Very Viscous	No	Non-inflammatory	None Remains
148	TKA	4.5 mL	Chunky	No	Septic	None Remains
149	TKA	3.5 mL	None	No	Non-inflammatory	None Remains
150	TKA	5.5 mL	Very Very Viscous	No	Non-inflammatory	None Remains
151	TKA	2.5 mL	None	No	Non-inflammatory	None Remains
152	TKA	5.5 mL	Bilateral - see 153	No	Non-inflammatory	None Remains
153	TKA	7.5 mL	Bilateral - see 152	No	Non-inflammatory	Microcentrifuge Tube
154	TKA	1.5 mL	Yellow, clear	No	Non-inflammatory	None Remains
155	TKA	7.5 mL	Some precipitate, not very viscous	No	Septic	None Remains
156	TKA	9.0 mL	Same - bilateral with 155	No	Septic	None Remains
157	TKA	3.5 mL	Orange	No	Hemarthrosis	None Remains
158	TKA	15.0 mL	Deep yellow with some precipitate	No	Inflammatory	None Remains
159	TKA	5.5 mL	Light yellow with little precipitate	No	Non-inflammatory	None Remains
160	Revision	4.0 mL	Pink	No	Hemarthrosis	None Remains
161	Revision	3.5 mL	Yellow	No	Non-inflammatory	None Remains
162	Revision	1.5 mL	Yellow, clear	No	Inflammatory	None Remains
163	Revision	9.0 mL	None	No	Non-inflammatory	None Remains
164	Revision	2.0 mL	Insufficient for viscometry	No	NR	None Remains
165	Revision	4.0 mL	Yellow, viscous	No	Non-inflammatory	None Remains
166	Revision	4.5 mL	None	No	NR	None Remains
167	Uni Rev	10.0 mL	Yellow with floating chunks & precipitate	No	Septic	Microcentrifuge Tube
168	Revision	8.0 mL	Pink, with red chunk	No	Hemarthrosis	1 MCF and 1 CF Tube

NR = Not reported or not recorded

+ Not all fluid from joint was recovered.

*Destroyed in cleanup, March, 2002

Appendix F: Description of Joint Fluid Samples

Study ID #	Occasion	+Quantity	Additional Gross Description	Blood ?	Gross Category	Location of remainder
169	Revision	2.5 mL	Plain Yellow	No	Non-inflammatory	None Remains
170	Revision	3.0 mL	Plain Yellow	No	Non-inflammatory	None Remains
171	TKA-e	3.0 mL	Deep Yellow with clump of Red Cells	Yes	Inflammatory	None Remains
172	Revision	11.5 mL	Viscous @ low shear, some red cells	Yes	Non-inflammatory	6 MCF and 1 CF Tube
173	Revision	1.5 mL	Yellow	No	Non-inflammatory	*None Remains
174	Revision	5.0 mL	Yellow	No	Non-inflammatory	Microcentrifuge Tube
175	Revision	4.5 mL	Watery, yellow with some red cells	No	Inflammatory	*None Remains
176	TKA-e	16.5 mL	Clear, yellow	No	Normal	2 Microcentrifuge Tubes
177	Revision	7.0 mL	Pink	No	Hemarthrosis	2 Microcentrifuge Tubes
178	Revision	3.0 mL	Yellow	No	Normal	3 Microcentrifuge Tubes
6*	TKA	NR	None	No	NR	3 Microcentrifuge Tubes
B	TKA	NR	None	No	NR	4 Microcentrifuge Tubes
H01	TKA	10.0 mL	Yellow, some opaque curls	No	Septic	CF and 4 MCF
H02	TKA	14.5 mL	Yellow, inviscid	No	Inflammatory	CF and 2 MCF
H03	TKA	7.0 mL	Bloody, with large clot	Yes	Bloody	*None Remains
H04	TKA	4.0 mL	Bloody and viscous	Yes	Bloody	*None Remains
H05	TKA	5.0 mL	Yellow	No	Non-inflammatory	None Remains
H06	TKA	1.5 mL	Pink	No	Hemarthrosis	*None Remains
H07	TKA	9.5 mL	Some precipitated bits	No	Non-inflammatory	*None Remains
H08	TKA	14.5 mL	None	No	Non-inflammatory	*None Remains
H09	TKA	8.5 mL	Opaque to writing, some redness	Yes	Inflammatory	2 Microcentrifuge Tubes
H11	TKA	12.5 mL	Yellow	No	Normal	*None Remains
H12	TKA	10.5 mL	Bloody, with some precipitate	Yes	Non-inflammatory	*None Remains
H13	TKA	10.0 mL	Yellow, some precipitate	No	Non-inflammatory	*None Remains
H14	TKA	9.5 mL	Pink	No	Hemarthrosis	*None Remains
H15	TKA	15.0 mL	Pink, some RBC's	No	Hemarthrosis	*None Remains
H16	TKA	10.0 mL	Yellow w/ red swirls	Yes	Inflammatory	CF and 2 MCF
H17	TKA	13.0 mL	Yellow, some aggregation	No	Non-inflammatory	CF and 2 MCF
H18	TKA	12.0 mL	Yellow	No	Non-inflammatory	CF and 2 MCF
H19	TKA	8.5 mL	Yellow, some aggregation	No	Non-inflammatory	2 Microcentrifuge Tubes
H20	TKA	14.5 mL	Translucent yellow	No	Inflammatory	CF and 2 MCF
H21	TKA	4.5 mL	Red	No	Hemarthrosis	CF and 2 MCF
H22	TKA	8.5 mL	Yellow w/ red swirls	Yes	Non-inflammatory	CF and 2 MCF
H23	TKA	13.0 mL	Yellow w/ pink, opaque swirls	No	Inflammatory	CF and 3 MCF
NR	Effusion	2.5 mL	Yellow, some aggregation, Insufficient	No	Non-inflammatory	None Remains
NR	TKA	NR	Damaged in shipping	No	Non-inflammatory	None Remains
NR	TKA	NR	Damaged in shipping	No	NR	None Remains

NR = Not reported or not recorded

+ Not all fluid from joint was recovered.

*Destroyed in cleanup, March, 2002

Appendix G: Viscous Properties of Joint Fluid

Study ID #	Occasion	Tester	Test Date	Cal.	Geometry	η_{1Pa}	ln η_{1Pa}	η_{1Pa} Range	ln η_0	η_0	η_{Max}	c [s]	d	Fit	η_0 Range
003	Revision-W	NER	9/19/00	1.00	Couette	0.033	-3.40	Degenerative	-0.35	0.706	0.063	93.92	0.381	Power	Degenerative
006	TKA	DCM	6/30/00	1.00	Cone	0.317	-1.15	Degenerative	0.19	1.212	0.799	2.04	0.595	Cross	Degenerative
007	TKA	DCM	7/12/00	1.00	Cone	2.602	0.96	Normal	1.94	6.957	4.976	5.74	0.641	Cross	Normal
009	TKA	NER	7/13/00	1.00	Cone	11.400	2.43	Normal	3.24	25.442	11.40	14.96	0.732	Cross	Normal
010	TKA	NER	7/17/00	1.00	Cone	5.461	1.70	Normal	2.20	9.044	7.869	3.40	0.575	Cross	Normal
011	TKA	NER	7/18/00	1.00	Cone	0.294	-1.22	Degenerative	0.40	1.487	0.926	3.53	0.576	Cross	Degenerative
012	Effusion	NER	10/29/00	0.86	Couette	0.070	-2.66	Degenerative	-0.50	0.607	0.361	4.32	0.499	Cross	Degenerative
013	Effusion	NER	7/21/00	1.00	Cone	0.123	-2.10	Degenerative	-0.34	0.710	0.241	2.19	0.530	Cross	Degenerative
015	Effusion	NER	7/21/00	1.00	Cone	0.039	-3.25	Degenerative	-1.32	0.267	0.069	2.25	0.874	Power	Degenerative
016	Effusion	DCM	7/20/00	1.00	Couette	0.180	-1.71	Degenerative	-0.37	0.688	0.464	1.26	0.535	Cross	Degenerative
018	TKA	NER	9/19/01	1.00	Couette	0.381	-0.97	Degenerative	1.51	4.515	1.544	28.82	0.578	Cross	Normal
019	Revision-W	NER	9/26/00	0.86	Couette	0.070	-2.66	Degenerative	-1.18	0.308	0.182	0.82	0.499	Cross	Degenerative
025	TKA	DCM	9/15/00	0.86	Couette	0.017	-4.07	Inflamed	-1.29	0.275	0.047	11.91	0.405	Power	Degenerative
028	TKA	NER	9/22/00	1.00	Couette	0.049	-3.01	Degenerative	1.31	3.701	0.064	1256.60	0.436	Power	Normal
030	TKA-E	DCM	9/22/00	1.00	Couette	0.183	-1.70	Degenerative	0.99	2.697	0.854	37.44	0.483	Cross	Normal
031	TKA	DCM	11/29/00	0.83	Couette	0.182	-1.70	Degenerative	-0.27	0.765	0.441	1.66	0.534	Cross	Degenerative
032	TKA	NER	10/26/00	0.86	Couette	0.020	-3.89	Inflamed	-1.71	0.182	0.118	2.52	0.429	Cross	Degenerative
033	TKA	NER	10/29/00	0.86	Couette	0.009	-4.67	Inflamed	-1.27	0.281	0.041	34.76	0.410	Power	Degenerative
034	TKA	NER	10/12/00	0.86	Couette	0.154	-1.87	Degenerative	0.95	2.597	0.954	19.02	0.592	Cross	Normal
035	TKA	NER	10/25/00	0.86	Couette	0.049	-3.01	Degenerative	0.03	1.028	0.309	18.57	0.506	Power	Degenerative
037	TKA	DCM	11/28/00	0.86	Couette	0.043	-3.16	Degenerative	-2.44	0.087	0.075	0.05	0.466	Cross	Degenerative
040	TKA	DCM	11/30/00	0.86	Couette	0.257	-1.36	Degenerative	-0.02	0.976	0.626	1.69	0.561	Cross	Degenerative
042	TKA	NER	10/14/00	0.86	Couette	0.066	-2.72	Degenerative	-0.49	0.610	0.326	4.45	0.505	Cross	Degenerative
044	TKA	NER	10/14/00	0.86	Couette	0.026	-3.65	Inflamed	0.56	1.749	0.159	142.28	0.487	Power	Degenerative
045	TKA	NER	10/14/00	0.86	Couette	0.045	-3.10	Degenerative	-1.01	0.363	0.174	2.68	0.483	Cross	Degenerative
046	TKA	NER	10/30/00	0.86	Couette	0.149	-1.90	Degenerative	0.15	1.163	0.689	3.86	0.586	Cross	Degenerative
047	Revision-N	NER	10/25/00	0.86	Couette	0.023	-3.78	Inflamed	-0.49	0.613	0.223	5.40	0.591	Cross	Degenerative
048	Effusion	NER	8/8/00	1.00	Cone	0.111	-2.19	Degenerative	-0.85	0.426	0.171	0.85	0.514	Cross	Degenerative
142	TKA	DCM	11/29/00	0.83	Couette	2.576	0.95	Normal	1.84	6.306	4.814	4.47	0.661	Cross	Normal
143	TKA	DCM	11/30/00	0.86	Couette	0.620	-0.48	Degenerative	0.78	2.186	1.577	2.78	0.641	Cross	Degenerative
144	TKA	DCM	11/30/00	0.86	Couette	0.040	-3.22	Degenerative	-1.61	0.199	0.088	2.38	0.356	Cross	Degenerative
145	TKA	DCM	11/30/00	0.86	Couette	0.308	-1.18	Degenerative	0.30	1.350	0.946	2.33	0.611	Cross	Degenerative
146	TKA	DCM	12/1/00	0.83	Couette	0.324	-1.13	Degenerative	0.52	1.689	0.100	345.48	0.423	Power	Degenerative
147	TKA	DCM	12/1/00	0.83	Couette	3.172	1.15	Normal	2.20	8.982	7.858	7.48	0.674	Cross	Normal
148	TKA	DCM	12/1/00	0.83	Couette	0.073	-2.62	Degenerative	0.07	1.069	0.126	19.69	0.466	Power	Degenerative
149	TKA	DCM	12/1/00	0.83	Couette	0.075	-2.58	Degenerative	-0.39	0.678	0.293	4.94	0.499	Cross	Degenerative
150	TKA	DCM	12/1/00	0.83	Couette	1.661	0.51	Degenerative	1.25	3.482	2.543	1.75	0.650	Cross	Normal
151	TKA	DCM	12/4/00	0.98	Couette	0.263	-1.34	Degenerative	0.98	2.677	1.417	12.73	0.578	Cross	Normal

In revision cases, W = wear-related failure; N = failure unrelated to wear

All viscosities are given in [Pa s]

Appendix G: Viscous Properties of Joint Fluid

Study ID #	Occasion	Tester	Test Date	Cal.	Geometry	η_{1Pa}	ln η_{1Pa}	η_{1Pa} Range	ln η_0	η_0	η_{Max}	c [s]	d	Fit	η_0 Range
152	TKA	DCM	12/4/00	0.98	Couette	0.443	-0.82	Degenerative	0.58	1.783	1.232	2.78	0.612	Cross	Degenerative
153	TKA	DCM	12/4/00	0.98	Couette	1.694	0.53	Degenerative	1.79	5.987	4.532	7.17	0.674	Cross	Normal
155	TKA	DCM	12/5/00	1.04	Couette	0.052	-2.96	Degenerative	-2.02	0.133	0.094	0.13	0.485	Cross	Degenerative
156	TKA	DCM	12/5/00	1.04	Couette	0.407	-0.90	Degenerative	0.66	1.927	1.295	3.86	0.584	Cross	Degenerative
158	TKA	DCM	12/5/00	1.04	Couette	0.036	-3.33	Degenerative	-1.01	0.365	0.066	12.34	0.381	Power	Degenerative
159	TKA	DCM	12/5/00	1.04	Couette	1.624	0.48	Degenerative	1.67	5.286	4.080	6.04	0.642	Cross	Normal
160	Revision-W	DCM	12/6/00	0.86	Couette	0.767	-0.27	Degenerative	1.37	3.952	2.532	8.66	0.597	Cross	Normal
161	Revision-W	DCM	12/6/00	0.86	Couette	0.173	-1.76	Degenerative	-0.35	0.703	0.426	1.48	0.534	Cross	Degenerative
163	Revision-N	DCM	2/7/01	1.10	Couette	0.306	-1.19	Degenerative	0.30	1.343	1.053	3.38	0.528	Cross	Degenerative
165	Revision-N	DCM	2/28/01	0.80	Couette	0.416	-0.88	Degenerative	0.43	1.532	1.027	2.47	0.568	Cross	Degenerative
167	Uni Rev	DCM	6/27/01	0.91	Couette	0.314	-1.16	Degenerative	0.30	1.346	0.898	2.72	0.558	Cross	Degenerative
168	Revision-W	DCM	6/27/01	0.91	Couette	0.016	-4.11	Inflamed	-2.78	0.062	0.034	0.23	0.385	Cross	Inflamed
169	Revision-W	DCM	8/1/01	0.94	Couette	0.212	-1.55	Degenerative	0.42	1.524	0.834	5.79	0.555	Cross	Degenerative
170	Revision-N	DCM	8/2/01	0.94	Couette	0.109	-2.22	Degenerative	1.50	4.482	0.268	196.18	0.494	Power	Normal
171	TKA-E	DCM	9/25/01	0.82	Couette	0.010	-4.64	Inflamed	-2.12	0.120	0.021	45.19	0.288	Power	Degenerative
172	Revision-W	DCM	10/15/01	1.05	Couette	0.143	-1.95	Degenerative	0.40	1.489	0.452	10.80	0.517	Power	Degenerative
174	Revision-N	DCM	11/1/01	0.90	Couette	0.013	-4.35	Inflamed	-3.15	0.043	0.024	0.13	0.364	Cross	Inflamed
175	Revision-N	DCM	11/26/01	1.11	Couette	0.004	-5.44	Inflamed	-4.75	0.009	0.005	0.00	0.207	Cross	Inflamed
176	TKA-E	DCM	3/21/02	0.79	Couette	0.007	-4.90	Inflamed	-1.12	0.326	0.033	58.69	0.414	Power	Degenerative
177	Revision-W	DCM	4/11/02	0.87	Couette	0.256	-1.36	Degenerative	0.50	1.651	0.990	6.18	0.539	Cross	Degenerative
H01	TKA	DCM	9/27/01	0.90	Couette	0.034	-3.37	Degenerative	-1.76	0.172	0.101	0.46	0.532	Cross	Degenerative
H02	TKA	DCM	10/4/01	1.07	Couette	0.022	-3.83	Inflamed	-2.40	0.091	0.054	0.21	0.517	Cross	Degenerative
H03	TKA	DCM	11/1/01	0.90	Couette	0.237	-1.44	Degenerative	0.73	2.079	1.132	9.58	0.560	Cross	Degenerative
H04	TKA	DCM	11/16/01	0.85	Couette	16.625	2.81	Normal	4.32	75.54	38.31	91.33	0.699	Cross	Normal
H05	TKA	DCM	11/26/01	1.11	Couette	1.470	0.39	Degenerative	1.49	4.429	3.644	4.61	0.639	Cross	Normal
H07	TKA	DCM	12/12/01	0.83	Couette	0.056	-2.89	Degenerative	4.97	143.9	0.792	991380	0.466	Power	Normal
H08	TKA	DCM	12/12/01	0.83	Couette	0.104	-2.26	Degenerative	0.01	1.010	0.478	6.21	0.508	Cross	Degenerative
H09	TKA	DCM	3/21/02	0.79	Couette	0.924	-0.08	Degenerative	1.61	5.020	2.394	11.02	0.607	Cross	Normal
H11	TKA	DCM	2/25/02	0.79	Couette	0.103	-2.27	Degenerative	-0.84	0.430	0.255	1.16	0.486	Cross	Degenerative
H12	TKA	DCM	2/25/02	0.79	Couette	0.025	-3.70	Inflamed	-1.30	0.273	0.084	5.02	0.431	Cross	Degenerative
H13	TKA	DCM	2/25/02	0.79	Couette	0.478	-0.74	Degenerative	0.59	1.809	1.301	2.91	0.571	Cross	Degenerative
H14	TKA	DCM	2/25/02	0.79	Couette	1.184	0.17	Degenerative	1.52	4.575	3.458	6.17	0.650	Cross	Normal
H15	TKA	DCM	2/25/02	0.79	Couette	0.123	-2.09	Degenerative	-0.46	0.631	0.363	1.78	0.534	Cross	Degenerative
H17	TKA	DCM	4/10/02	0.90	Couette	0.049	-3.02	Degenerative	-1.36	0.256	0.118	1.91	0.397	Cross	Degenerative
H18	TKA	DCM	4/10/02	0.90	Couette	0.250	-1.38	Degenerative	0.47	1.606	0.965	6.07	0.538	Cross	Degenerative
H19	TKA	DCM	4/11/02	0.87	Couette	0.106	-2.25	Degenerative	5.97	391.8	3.037	402550	0.536	Power	Normal
H20	TKA	DCM	4/11/02	0.87	Couette	0.940	-0.06	Degenerative	2.25	9.495	5.422	44.58	0.579	Cross	Normal
H23	TKA	DCM	8/9/02	0.90	Couette	0.047	-3.05	Degenerative	-1.20	0.301	0.127	3.55	0.392	Cross	Degenerative

In revision cases, W = wear-related failure; N = failure unrelated to wear

All viscosities are given in [Pa s]

Appendix H: Summary of Viscoelastic Parameters of Each Joint Fluid Sample

Study ID #	Occasion	Tester	Test Date	Cal.	f_c [rad/sec]	G_c	X-over?	$G'_{0.5Hz}$	$G''_{0.5Hz}$	$G'_{2.5Hz}$	$G''_{2.5Hz}$	G'_{5Hz}	G''_{5Hz}
016	Effusion	DCM	7/20/00	1.00	70.90	2.31	Yes	0.358	0.555	1.066	1.218	1.570	1.633
019	Revision-W	NER	9/26/00	0.86	> 59	No X	No	0.115	0.265	0.455	0.685	0.697	0.979
024	TKA	NER	9/29/00	0.86	20.75	0.34	Yes	0.543	0.102	0.219	0.273	0.521	0.424
025	TKA	DCM	9/15/00	0.86	> 33	No X	No	0.280	0.102	0.209	0.341	0.293	0.486
029	TKA	NER	9/29/00	0.86	19.01	0.48	Yes	0.160	0.262	0.400	0.440	0.816	0.624
031	TKA	DCM	11/29/00	0.83	34.79	1.48	Yes	0.376	0.518	1.047	1.086	1.426	1.415
034	TKA	NER	10/12/00	0.86	3.44	0.44	Yes	0.416	0.426	0.844	0.667	1.248	0.854
040	TKA	DCM	11/30/00	0.86	14.68	1.18	Yes	0.436	0.606	1.223	1.213	1.697	1.578
045	TKA	NER	10/14/00	0.86	24.88	0.64	Yes	N/A	N/A	0.345	0.471	0.819	0.738
142	TKA	DCM	11/29/00	0.83	1.79	1.72	Yes	2.354	2.080	4.957	3.207	6.360	3.735
144	TKA	DCM	11/30/00	0.86	> 56	No X	No	N/A	N/A	0.201	0.490	0.375	0.792
145	TKA	DCM	11/30/00	0.86	5.52	0.85	Yes	0.633	0.710	1.522	1.196	1.958	1.435
147	TKA	DCM	12/1/00	0.83	1.46	1.62	Yes	2.632	2.080	5.194	2.909	6.437	3.267
150	TKA	DCM	12/1/00	0.83	5.22	2.56	Yes	1.835	2.093	4.610	3.636	6.238	4.399
152	TKA	DCM	12/4/00	0.98	4.53	1.00	Yes	0.826	0.884	1.924	1.502	2.461	1.831
153	TKA	DCM	12/4/00	0.98	1.22	1.11	Yes	1.888	1.468	3.640	2.067	4.414	2.360
156	TKA	DCM	12/5/00	1.04	7.16	1.23	Yes	0.721	0.888	1.838	1.577	2.490	1.981
161	Revision-W	DCM	12/6/00	0.86	39.14	1.56	Yes	0.337	0.525	0.981	1.072	1.424	1.430
163	Revision-N	DCM	2/7/01	1.10	17.83	2.14	Yes	0.000	0.000	1.512	1.541	2.218	2.292
165	Revision-N	DCM	2/28/01	0.80	24.84	1.58	Yes	0.742	0.935	1.959	1.850	2.620	2.336
167	Uni Rev	DCM	6/27/01	0.91	14.85	1.71	Yes	0.620	0.870	1.736	1.730	2.508	2.276
168	Revision-W	DCM	6/27/01	0.91	> 43	No X	No	N/A	N/A	0.085	0.298	0.120	0.482
169	Revision-W	DCM	8/1/01	0.94	6.42	0.82	Yes	0.541	0.619	1.294	1.142	1.728	1.445
170	Revision	DCM	8/2/01	0.94	13.65	0.83	Yes	0.355	0.451	0.897	0.871	1.188	1.109
171	TKA-E	DCM	9/25/01	0.82	> 79	No X	No	N/A	N/A	0.054	0.193	0.141	0.296
172	Revision-W	DCM	10/15/01	1.05	19.63	1.25	Yes	0.468	0.633	1.192	1.222	1.678	1.583
174	Revision-N	DCM	11/1/01	0.90	> 49	No X	No	N/A	N/A	0.073	0.226	0.074	0.404
175	Revision-N	DCM	11/26/01	1.11	None	No X	No	0.000	0.020	N/A	N/A	N/A	N/A
176	TKA-E	DCM	3/21/02	0.79	> 79	No X	No	0.010	0.037	0.049	0.129	0.083	0.209
177	Revision-W	DCM	4/11/02	0.87	10.88	1.19	Yes	0.569	0.697	1.444	1.383	1.959	1.813

In revision cases, W = wear-related failure; N = failure unrelated to wear
No X = no viscoelastic crossover

All moduli are given in units of [Pa]

Appendix H: Summary of Viscoelastic Parameters of Each Joint Fluid Sample

Study ID #	Occasion	Tester	Test Date	Cal.	f_c [rad/sec]	G_c	X-over?	$G'_{0.5Hz}$	$G''_{0.5Hz}$	$G'_{2.5Hz}$	$G''_{2.5Hz}$	G'_{5Hz}	G''_{5Hz}
H01	TKA	DCM	9/27/01	0.90	> 62	No X	No	N/A	N/A	0.324	0.426	0.447	0.565
H02	TKA	DCM	10/4/01	1.07	> 79	No X	No	N/A	N/A	0.211	0.300	0.322	0.411
H03	TKA	DCM	11/1/01	0.90	5.03	0.87	Yes	0.660	0.726	1.533	1.280	1.981	1.587
H04	TKA	DCM	11/16/01	0.85	0.34	2.71	Yes	7.941	4.495	9.903	5.213	11.545	5.525
H05	TKA	DCM	11/26/01	1.11	3.57	1.95	Yes	1.818	1.861	4.194	2.998	5.489	3.547
H07	TKA	DCM	12/12/01	0.83	> 79	No X	No	0.209	0.414	0.669	0.902	0.934	1.203
H08	TKA	DCM	12/12/01	0.83	34.83	1.32	Yes	0.302	0.452	0.894	0.965	1.247	1.275
H09	TKA	DCM	3/21/02	0.79	2.27	1.07	Yes	1.311	1.199	2.779	1.912	3.557	2.268
H11	TKA	DCM	2/25/02	0.79	> 79	No X	No	0.192	0.384	0.691	0.960	1.016	1.345
H12	TKA	DCM	2/25/02	0.79	> 79	No X	No	N/A	N/A	0.276	0.412	0.397	0.580
H13	TKA	DCM	2/25/02	0.79	6.95	1.28	Yes	0.793	0.929	1.994	1.759	2.675	2.216
H14	TKA	DCM	2/25/02	0.79	1.78	1.02	Yes	1.445	1.243	2.916	1.830	3.649	2.105
H15	TKA	DCM	2/25/02	0.79	56.98	1.53	Yes	0.305	0.439	0.854	0.916	1.177	1.212
H17	TKA	DCM	4/10/02	0.90	> 62	No X	No	0.075	0.214	0.345	0.647	0.536	0.957
H18	TKA	DCM	4/10/02	0.90	13.59	1.22	Yes	0.483	0.645	1.319	1.294	1.760	1.655
H20	TKA	DCM	4/11/02	0.87	2.85	1.47	Yes	1.569	1.529	3.150	2.354	4.000	2.753
H23	TKA	DCM	8/9/02	0.90	> 124	No X	No	0.073	0.211	0.324	0.618	0.511	0.937

In revision cases, W = wear-related failure; N = failure unrelated to wear
No X = no viscoelastic crossover

All moduli are given in units of [Pa]

APPENDIX I:
STANDARD SPECTROPHOTOMETRIC CURVES

I.1 Protocol for Standard Curve of Protein Concentration

1. Dilute 50 µl bovine serum albumin (BSA) stock solution, which is kept frozen at 1.5 µg/µl, at a 1:9 ratio with deionized water (dH_2O), making a 0.15 µg/µl solution.
2. Prepare twelve mixtures for the standard curve, using the table as a guide. Each preparation totals 2000 µl. Prepare each standard in duplicate.

Cell #	*BSA volume [µl]*	*Protein Content [µg]*	*dH_2O [µl]*	*Bio-Rad Dye [µl]*
1	0	0	1600	400
2	10	1.5	1590	400
3	20	3	1580	400
4	40	6	1560	400
5	60	9	1540	400
6	80	12	1520	400

I.2 Protocol for Standard Curve of Phospholipid Concentration

1. Dilute 800 µl Phospholipids B Standard (PS) Stock Solution (3.00 µg/µl), which is kept at 2-10°C, at a 1:1 ratio with dH_20 to make a 1.50 µg/µl solution.
2. Prepare eight mixtures for the standard curve, using the chart below as a guide. Each preparation totals 3000 µl. Prepare each standard in duplicate.

Cell #	*PS Volume [µl]*	*Phospholipid Content [µg]*	*dH_20 [µl]*	*Phospholipids B Color Reagent [µl]*
1	0	0	2000	1000
2	10	15.0	1990	1000
3	20	30.0	1980	1000
4	40	60.0	1960	1000

I.3 How to Handle Samples for Spectrophotometry

1. Each sample is to be added into two-sided clear plastic cuvettes. Cuvettes should be handled only above the reading line, and only on the ridged sides, never on the clear sides.
2. The blank is always to be read first, followed by five samples.
3. The standard curve is to be generated using the slope of the line $y = mx + b$, where y is the protein or phospholipid content in the sample, x is the absorbance reading, and m and b are constants which best fit a linear standard curve.

The protein concentration in the original synovial fluid sample can be found by dividing the protein content by 0.25 µl, the total volume of synovial fluid used. The phospholipid concentration in the original synovial fluid sample can be found by dividing the measured phospholipid content by 50 µl, the volume of synovial fluid used.

APPENDIX J
CALIBRATION OF SEC DATA

J.1 Confirmation of HA Concentration

To demonstrate that the total mass of HA present was proportional to the area under the refractive index curve, the carbazole reaction was performed on three aliquots of three joint fluid samples. The relationship between concentration of HA, as determined by the carbazole reaction, and area under the refractive index curve in SEC is given below in Fig. J.1. Based on the goodness of fit of a line through the origin in this relationship, the use of SEC in lieu of the carbazole reaction is appropriate for determination of HA concentration in joint fluid samples.

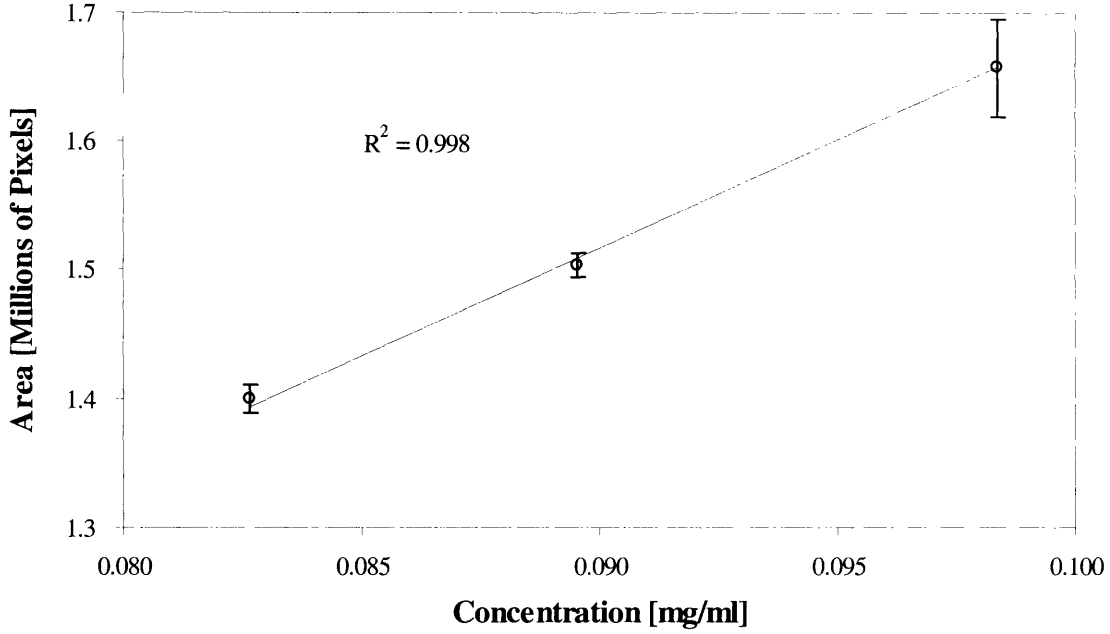


Fig J.1 HA concentration by SEC versus by carbazole reaction Using three samples, a linear relationship is demonstrated between HA concentration as determined by carbazole reaction (x-axis) and SEC (y-axis). The best fit line through the origin is shown, with coefficient of determination.

J.2 Calibration of HA Molecular Weight

Elution of standard HA preparations are given below in Fig. J.2. Each standard was eluted twice in the final calibration, and these calibrations were interspersed with joint fluid samples during the course of experimentation. Only one curve for each molecular weight is shown in Fig. J.2. These standards demonstrated a linear relationship between the logarithm of M_v and the peak elution time up to 1.7 MDa as shown in Fig. J.3 ($R^2 = 0.96$). A function was calculated using the method of least squares, such that $M_v = 7.39 \times 10^{(9 - 0.535t)}$, where t is the peak elution time in minutes. Based on this relationship, M_p was calculated for each sample. Most samples exhibited M_p greater than 1.7 MDa, but the highest value was 2.0 MDa, so although extrapolation of the calibration curve was necessary, it was not necessary beyond the known operating range of the column (previous calibrations had demonstrated the range of the column up to 2.0 MDa).

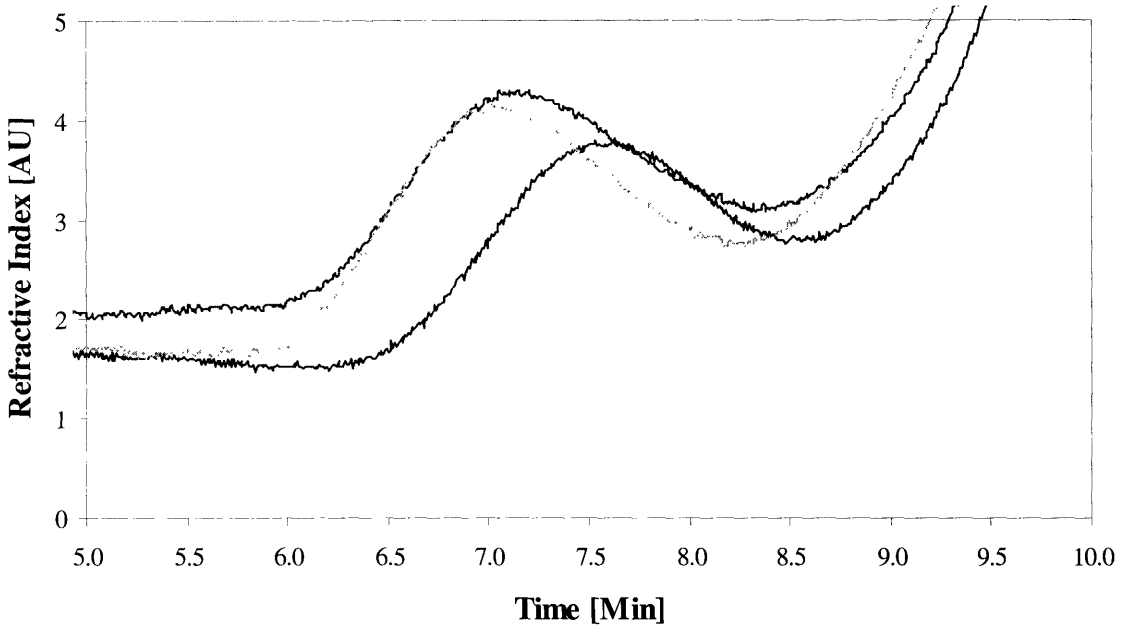


Fig J.2 Elution of HA standards by refractive index measurement The black line represents elution of a 0.786 MDa standard. The dark gray line represents elution of a 1.26 MDa standard, the gray line represents elution of 1.68 MDa standards, and the light gray line represents elution of a 2.7 MDa standard.

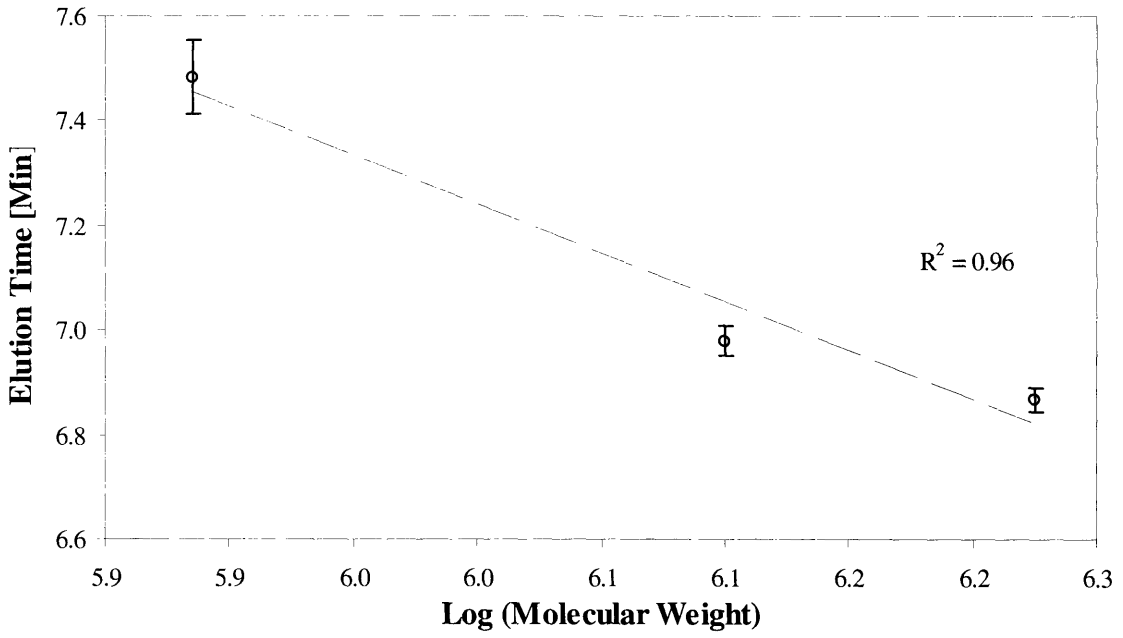


Fig J.3 Elution time of HA standards versus molecular weight The three samples exhibit an inverse relationship between elution time and molecular weight up to 1.68 MDa. 2.7 MDa standard is not included in this figure. Bars represent standard deviation.

Only one sample of the 2.7 MDa standard (part number 1782-05, lot number 762602, Genzyme, Cambridge, MA) was eluted, and its output was not consistent with the relationship demonstrated by the others (Fig. J.4). Although it may have been due to saturation in column behavior at high molecular weight, there are other less obvious but

more likely explanations, as described below in section J.3. Therefore, the performance of the column is assumed linear throughout the operating range, as described in the text of Chapter 4.

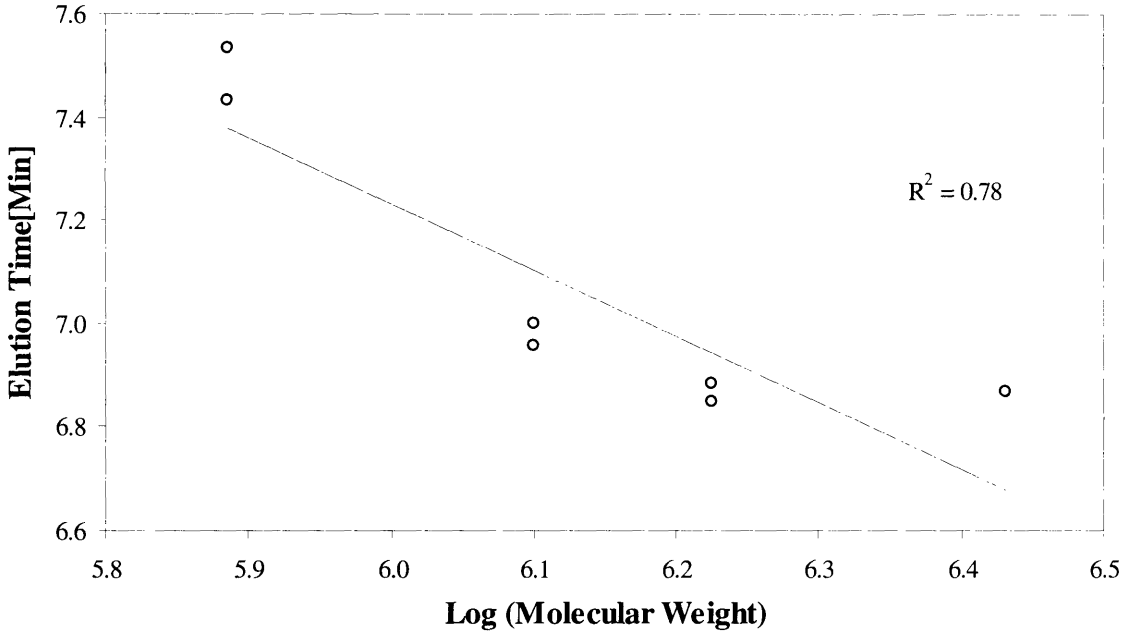


Fig J.4 Elution time of HA standards versus molecular weight (reprise) In this figure, the individual measurements are shown, including the 2.7 MDa standard.

J.3 Sources of Error in HA Molecular Weight Determination

The data presented in this appendix suggest that retention time may saturate at molecular weights close to that of the highest HA standard. In that case, the data for HA molecular weight presented in Chapter 4 would underestimate the true spread of HA molecular weight in joint fluid. This is the most significant source of potential error in this study, and it casts doubt on the narrowness of molecular weight distribution reported in this thesis. For this reason, it is suggested that future work employ the set of columns used for initial studies (cf. section 4.4.4) rather than those used in Chapter 4.

It should be noted that the poor behavior of the high molecular weight standard has other explanations. For example, it may equally likely reflect the difficulty of obtaining good high molecular weight standards. Thus, the column may have separated the molecules appropriately, but the peak did not correspond well with M_v. If this were the case, no error would be introduced by evaluating HA molecular weight on this column.

By careful examination of HA standard elution time, an additional possible source for this discrepancy could be found in an upward drift of retention time during the course of the experiments. This drift was not caused by changes in flow rate, which were small. Drift can be demonstrated by examining the elution time of the many low molecular weight species in the standard. These eluted all at once after all larger molecules. During the course of the experiments, the elution time of these species increased slightly. Since the standards were evaluated from low molecular weight to high, interspersed with joint

fluid samples, the elution time of the high molecular weight sample was increased the most, reducing the linearity of the standard curve.

It was possible to try to correct for this error by one of two methods. One method involved converting the elution time to a dimensionless elution times based upon the elution time of low molecular weight species. By using a dimensionless time that is the ratio of these parameters, the linear relationship shown in Fig. J.4 improves ($R^2 = 0.93$). Alternatively, the elution time of HA could be calculated not based upon start time, but based upon the difference between its elution time and that of the low molecular weight species. Using this means of calculation, the linear relationship improves still further ($R^2 = 0.943$.) Based upon consideration of these alternate sources of the poor correlation of the high molecular weight species, it was deemed reasonable to exclude this high molecular weight standard in the elution time calculation.

In calculating the elution time of action joint fluid samples, a relative or dimensionless time cannot be calculated because many low molecular weight species are present, interfering with the output curve at the necessary times. Therefore, the error introduced by this drift could not be removed.

Additional sources of error in HA standards follow. Because the pump is pressure controlled, flow rate may vary with time based upon changes in column resistance. HA molecular weight may be affected by proteolytic degradation at high temperatures. The use of M_v as an estimate for M_p when determining the relationship between elution time and molecular weight may introduce some errors. Finally, viscous fingering may have an adverse effect at high concentration, whereas signal to noise ratio may have an effect at low concentration.

All these limitations may also have affected the real joint fluid samples as well as the standards. Furthermore, joint fluid sample evaluation may be somewhat affected by non-homogeneity in joint fluid samples. Finally, as mentioned above, the HA standards themselves are limited, in that they are not as monodisperse and well defined as one may prefer.

APPENDIX K
CALCULATION OF MOLECULAR WEIGHTS

When using SEC to determine concentration and molecular weight of species, parameters were calculated on a spreadsheet, using refractive index absorbance relative to baseline as described in section 4.4.3. M_n, M_w, and M_z were calculated using the following equations.

$$M_n = \frac{\sum AU}{\sum (AU/MW)}$$

$$M_w = \frac{\sum (AU \times MW)}{\sum AU}$$

$$M_z = \frac{\sum (AU \times MW^2)}{\sum (AU \times MW)}$$

In these equations, AU is refractive index absorbance at a given retention time, and MW is the molecular weight corresponding to a given retention time, based upon the elution of standards.

Concentration was calculated as the sum of absorbance over all molecular weights divided by a conversion factor. That factor was calculated to be 2.93×10^4 for the HA for the conditions of these experiments.

Appendix L: Summary of Composition of Each Joint Fluid Sample

Study ID #	Occasion	Phospholipid [mg/ml]	Protein [mg/ml]	M_n [MDa]	M_w [MDa]	M_z [MDa]	M_{z+1} [MDa]	Conc [mg/ml]	Polydispersity
003	Revision-W	0.589	47.3	1.562	1.267	1.617	1.997	0.624	1.276
006	TKA	0.738	47.4	1.861	1.437	1.791	2.158	1.216	1.247
009	TKA	0.295	27.0	2.049	1.499	1.927	2.370	2.107	1.285
011	TKA	0.541	30.1	NR	NR	NR	NR	NR	NR
012	Effusion	0.263	21.9	1.767	1.424	1.835	2.279	1.116	1.289
013	Effusion	0.310	15.4	1.653	1.320	1.711	2.131	1.169	1.296
015	Effusion	NR	NR	1.653	1.218	1.579	1.941	1.435	1.296
018	TKA	0.480	31.4	2.030	1.456	1.854	2.267	1.155	1.274
019	Revision-W	0.539	36.3	1.869	1.518	1.872	2.252	0.695	1.233
022	TKA	0.414	18.7	NR	NR	NR	NR	NR	NR
025	TKA	0.632	52.5	2.039	1.462	1.807	2.141	0.694	1.236
026	TKA	0.672	23.2	NR	NR	NR	NR	NR	NR
030	TKA-E	0.286	20.4	NR	NR	NR	NR	NR	NR
031	TKA	0.574	35.1	1.507	1.051	1.420	1.794	1.700	1.351
036	TKA	0.395	25.1	NR	NR	NR	NR	NR	NR
039	TKA	0.225	18.4	NR	NR	NR	NR	NR	NR
040	TKA	0.495	13.0	1.785	1.529	1.930	2.375	1.339	1.263
041	TKA	0.399	13.2	NR	NR	NR	NR	NR	NR
043	TKA	0.271	16.8	NR	NR	NR	NR	NR	NR
044	TKA	0.429	23.5	1.653	1.197	1.604	2.025	0.962	1.340
046	TKA	0.286	22.8	NR	NR	NR	NR	NR	NR
047	Revision-N	0.325	20.0	1.679	1.064	1.554	2.053	2.794	1.461
050	TKA	0.266	17.0	1.860	1.341	1.788	2.255	2.068	1.333
152	TKA	0.399	22.3	1.978	1.414	1.837	2.280	1.310	1.299
153	TKA	0.321	10.4	2.000	1.371	1.862	2.347	2.553	1.358
162	Revision-W	0.321	17.1	NR	NR	NR	NR	NR	NR
164	Revision-W	0.312	25.6	NR	NR	NR	NR	NR	NR
167	Uni Rev	0.263	11.0	1.644	1.167	1.553	1.929	1.834	1.330
168	Revision-W	0.497	26.9	1.571	1.165	1.483	1.816	1.184	1.274
169	Revision-W	0.295	27.9	NR	NR	NR	NR	NR	NR
170	Revision-N	0.331	14.2	NR	NR	NR	NR	NR	NR

TKA-E = Effusion after TKA

In revision cases, W = wear-related failure; N = failure unrelated to wear

NR = not recorded

Appendix L: Summary of Composition of Each Joint Fluid Sample

Study ID #	Indication	Phospholipid [mg/ml]	Protein [mg/ml]	M_w [MDa]	M_w [MDa]	M_w [MDa]	M_w [MDa]	Conc [mg/ml]	Polydispersity
171	TKA-E	0.624	NR	NR	NR	NR	NR	NR	NR
172	Revision-W	0.496	33.8	1.796	1.360	1.801	2.265	1.318	1.324
173	Revision-N	0.749	47.5	NR	NR	NR	NR	NR	NR
174	Revision-N	0.858	26.7	1.841	1.424	1.796	2.186	0.498	1.261
175	Revision-N	0.818	54.3	NR	NR	NR	NR	NR	NR
176	TKA-E	0.652	33.6	1.749	1.500	1.830	2.187	0.280	1.220
177	Revision-W	0.527	32.4	1.881	1.386	1.802	2.235	1.499	1.300
178	Revision-W	0.809	62.3	1.739	1.575	1.902	2.260	0.527	1.207
6*	TKA	0.472	17.5	1.823	1.294	1.741	2.212	1.815	1.345
B	TKA	0.585	34.6	1.832	1.321	1.686	2.031	1.078	1.276
H01	TKA	0.637	33.2	1.998	1.543	1.930	2.335	0.962	1.251
H02	TKA	0.343	14.6	1.786	1.356	1.731	2.129	0.729	1.277
H03	TKA	0.915	40.4	1.776	1.628	1.975	2.379	1.317	1.213
H04	TKA	0.261	18.7	NR	NR	NR	NR	NR	NR
H06	TKA	0.455	24.2	NR	NR	NR	NR	NR	NR
H07	TKA	0.342	25.4	NR	NR	NR	NR	NR	NR
H08	TKA	0.536	29.2	NR	NR	NR	NR	NR	NR
H09	TKA	0.681	32.6	1.937	1.469	1.894	2.335	1.726	1.289
H11	TKA	0.519	28.2	NR	NR	NR	NR	NR	NR
H12	TKA	0.544	33.9	NR	NR	NR	NR	NR	NR
H13	TKA	0.564	29.4	NR	NR	NR	NR	NR	NR
H14	TKA	0.338	25.4	NR	NR	NR	NR	NR	NR
H15	TKA	0.544	34.9	NR	NR	NR	NR	NR	NR
H16	TKA	0.601	26.3	1.950	1.349	1.813	2.299	1.457	1.344
H17	TKA	0.776	29.8	1.670	1.400	1.757	2.147	0.862	1.255
H18	TKA	0.637	25.9	1.850	1.454	1.824	2.217	1.382	1.254
H19	TKA	0.607	39.2	1.937	1.571	1.949	2.346	0.962	1.241
H20	TKA	0.522	31.1	1.969	1.393	1.843	2.319	1.707	1.324
H21	TKA	0.777	45.3	1.750	1.438	1.856	2.295	1.014	1.291
H22	TKA	0.787	38.8	1.707	1.384	1.779	2.196	1.253	1.285
H23	TKA	0.961	22.0	1.785	1.564	1.902	2.271	0.711	1.217

TKA-E = Effusion after TKA

In revision cases, W = wear-related failure; N = failure unrelated to wear

NR = not recorded

APPENDIX M
CALIBRATION OF FRICTION APPARATUS

Several means of calibration were attempted before the method was determined. Since I see no potential benefit to inclusion of the initial means of calibration, these preliminary methods, and their shortcomings, are excluded.

The calibration used for these experiments was as follows. With no normal load, the offset of the voltage output was set to zero. Then a load was applied via a weight applied to a pulley. The force was measured over the course of ten to twenty seconds. This was done for seven different loads, including zero. The mean voltage output was charted versus applied load after having been normalized by the zero load. A typical output graph is given below in Fig M.1

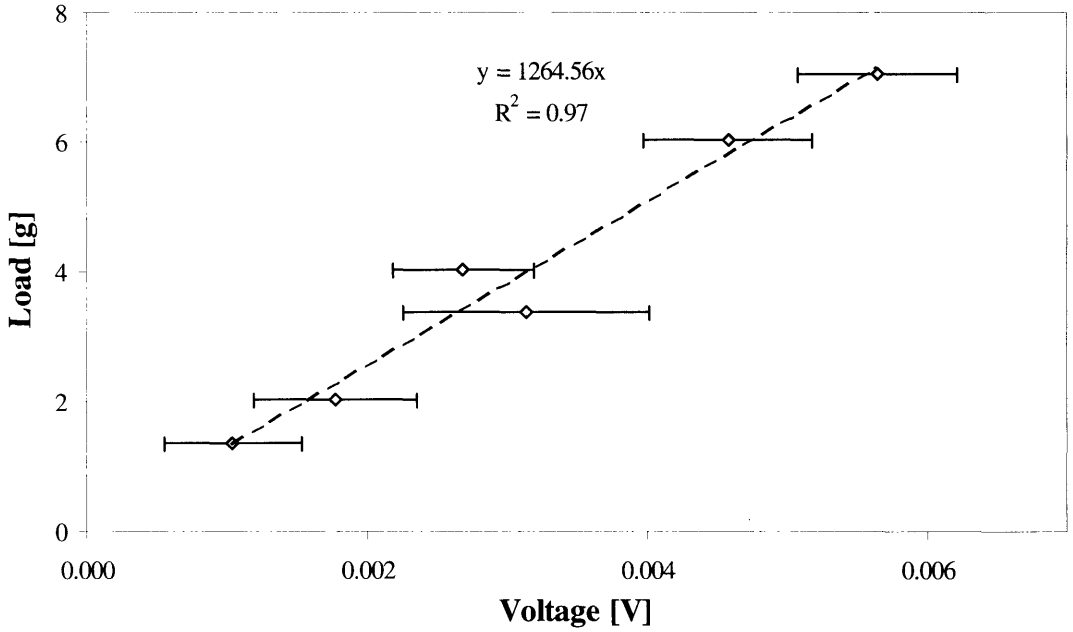


Fig. M.1 Typical calibration of friction apparatus This calibration was performed for the month of March, 2003. Error bars represent standard deviation. Since these loads are standardized against a load of 0 g, the line is fit through the origin.

Calibrations of this kind have been created for all months in which tests were performed. At the start of tests, calibrations were performed more often than monthly. This means of calibration is preferable to other means that employ bidirectional motion and perhaps is more scientific than the use of a standard lubricant at the start or end of each experiment as a baseline measure. The benefit of a bidirectional method is that any voltage offset in one direction would be cancelled by rotation in the other direction. The benefit of a standard lubricant is that it may remove a bias inherent in individual pins. On the other hand, it would reduce the number of non-control tests conducted per experiment. The standard lubricant method was discarded because a comparison of PE on Co-Cr revealed there was no effect of pin choice.

APPENDIX N
CALCULATION OF POF ARTICULATION PATTERNS

The path of PE pins articulating on Co-Cr disks were determined using numerical geometric analysis. The Ortho-POD wear tester was laid out as follows. Six cups were equally spaced at a distance 52.4 mm from the center of the large water bath. Each pin was attached to a pivot offset 25.4 mm from a point 52.4 mm from the center of the large water bath. The lubricant bath containing Co-Cr cups was attached to the bottom plate, which could rotate freely. The pivots could rotate freely (but not independently) to enable motion in a direction different than that of the disks. Figure N.1 shows the pins and disks in the default position, and with each pin is centered on a disk (bottom plate rotated clockwise 28.1° and pins rotated 76.0° counterclockwise).

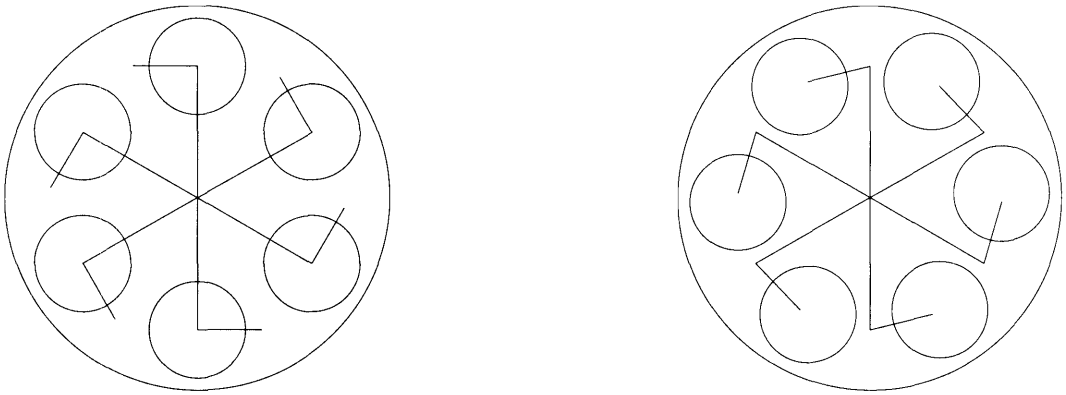


Fig. N.1 POD tester viewed from top Large circle represents the large lubricant batch. Small circles represent individual lubricant cups. The *l*-shaped lines emanating from the center hold PE pins on their ends. On the left is the default position, and on the right is with pins centered on the disks. This figure is drawn in 3:1 scale.

The square, 10 mm on a side, was traced in 16 points, such that five points were on each side of the square (with the four corner points being shared by two sides). Points are denoted as (disk rotation, pin rotation). The following pairs of rotations were used for the 16 points, with clockwise rotations being positive: first side {(6.9, -10.9); (6.2, -5.2); (5.4, 0.6); (4.3, 6.4); (3.0, 12.5)}; second side {(3.0, 12.5); (0.6, 12.1); (-1.8, 11.9); (-4.4, 12.1); (-6.9, 12.5)}; third side {(-6.9, 12.5); (-6.1, 6.4); (-5.5, 0.5); (-5.2, -5.1); (-5.2, -10.9)}; fourth side {(-5.2, -10.9); (-2.2, -11.3); (0.8, -11.5); (3.8, -11.3); (6.9, -10.9)}. For the friction measurements, a line was drawn across the center of each disk. The line was drawn using 10 points. The points in this line included: {(5.4, 0.5); (2.7, 0.1); (0.0, 0.0); (-2.8, 0.1); (-5.6, 0.5)} (the return path employed the same five points in the opposite order). Given 0.1° precision on disk rotation, the maximum error in disk rotation measurement is less than 0.09 mm. This was much smaller than the precision with which dimensions have been measured, and introduced no additional error.

APPENDIX O
STATISTICAL METHODS

O.1 Power Calculations

For ANOVA, Student's *t*-test, and analysis of covariance, power calculations were performed to determine the sample sizes necessary to detect significant differences between experimental groups. The sample sizes could be calculated as follows:

$$n = 2(\frac{\sigma}{\delta})^2 (t_{\alpha,\nu} + t_{2\beta,\nu}),$$

where n is sample size, σ is standard deviation, δ is the desired difference to detect, α is the desired significance level (probability of obtaining a false positive result), β is the desired statistical power (probability of obtaining a false negative result), $t_{\alpha,\nu}$ is the t statistic corresponding to a significance level α and ν degrees of freedom, and $t_{2\beta,\nu}$ is the t statistic corresponding to significance level 2β and ν degrees of freedom.

The solutions of this equation have been tabulated for various values of σ, δ, α, and β. The difference (δ) between groups that would be meaningful depended on the comparison. For experiments in which determining a difference between two groups was the primary aim, assumed meaningful differences and standard deviations were given under the heading "Statistical Methods." Using these values and setting the criteria for significance to be $\alpha = 0.05$ and $\beta = 0.2$ or some other value, samples sizes were determined for each group.

These analyses test the hypothesis that there is no difference in the mean values between two groups. It assumes that each group is normally distributed with the same variance. After evaluating the data from Chapter 3, it was found that these assumptions were not true, and such an analysis could not be used. In data from other chapters, these assumptions were not invalidated.

O.2 Mann-Whitney Test

The Mann-Whitney test was used to demonstrate a significant difference between two groups that were not normally distributed. This test compares the ranks of the two groups, rather than their actual values. The existence of a difference between two groups was calculated as follows:

$$Z = \frac{U - \frac{n_1 n_2}{2}}{\sqrt{\frac{n_1 n_2 (n_1 + n_2 + 1)}{12}}},$$

where n_1 is the number of samples in the first group and n_2 is the number of samples in the second group. U is *either* the sum, over each sample in the first group, of the number of members of the second group preceding it in rank *or* the sum, over each sample in the second group, of the number of members of the first group preceding it in rank, *whichever is less*. Z is the z-value determining the p-value for a two-tailed test, and therefore the probability of a false positive result.

This analysis tests the hypothesis that there is no difference in the range of values between two groups. It does not assume that each group is normally distributed.

O.3 Fisher's Exact Test

A two-tailed Fisher Exact test was also used to determine significant differences using a two by two matrix. An example of this calculation follows.

	X-over Measured	*No X-over Measured*	*Row Total*
$\eta_{1Pa} < a$	w	X	$R_1 = w + x$
$\eta_{1Pa} > a$	Y	Y	$R_2 = y + z$
Column Total	$C_1 = w + y$	$C_2 = x + z$	$N = w + x + y + x$

$$P_{crit} = \frac{(R_1!R_2!)(C_1!C_2!)}{N!(w!x!y!z!)}$$

and

$$p\text{-value} = \sum(P - values \leq P_{crit})$$

This test was used, for example, to demonstrate a relationship between viscosity and viscoelasticity, using a = 1 Pa s or a = 0.5 Pa s.